DIFFERENTIAL DIAGNOSIS OF CATARACTS BY CRYSTALLIN SUBUNIT QUANTITATION CORRELATED WITH CLINICAL AND MORPHOLOGICAL OBSERVATIONS.

John Cuthbert B.Sc. (Edinburgh)

Thesis presented in requirement for the degree of Doctor of Philosophy to University of Edinburgh, Departments of Ophthalmology and Animal Genetics.

1981.

I hereby declare that all of the analyses on the individual lenses described in this thesis have been performed by me. The collection of the data from patients and controls as part of an epidemiological study was carried out by members of the Department of Ophthalmology, the measurements of plasma constituents was carried out in the Department of Clinical Chemistry and the computer storage of the data organised in the Department of Statistics. The handling of the statistical results was carried out by me except where otherwise stated.

CONTENTS

SUMMARY

A large number of human cataractous lenses have been obtained for individual lens analysis. Classification and grouping of the lenses was initially based on the clinical description of both nuclear and cortical involvements made previous to surgical extraction. Measurements of the wet, dry and percentage dry weights were, in general, in agreement with those found in the literature and the relationship of these parameters to the stage of cataract is discussed. Iso-electric focussing of the water soluble (W.S.) and urea soluble (U.S.) fractions of the lenses yielded protein profile for each lens based on the levels of the individual polypeptide subunits as measured by densitometry. The profiles were found to reflect the nature of the lesion and, thus, the initial classification and grouping of the lenses was validated. Furthermore, comparisons made between the protein profiles of cataracts of similar morphopathology but differing aetiology - those considered here include diabetes, retinitis pigmentosa and leprosy - showed that the protein profile reflected not only the location and stage of the lesion within the lens but also the mechanism of cataractogenesis.

Computer analysis of information collected from a population of cataract patients and controls, concerning their social and medical backgrounds as well as their ophthalmic record and clinical chemistry record of blood plasma constituents, was carried out. A number of single variables were found to be associated with cataract incidence. They included the medical conditions; diabetes, cardiovascular disease, high blood pressure and other eye diseases; and the prolonged use of some drugs including the

tranquilisers, barbiturates, MAOI, tricyclics and phenothiazines, and steroids and miotics when applied topically to the eye. High levels of the plasma constituents urea and glucose and low levels of calcium, cholesterol, total protein, albumin, total CO_2, phosphate, alanine aminotransferase and asparagine aminotransferase are also found to associate with cataract incidence. A lower incidence of cataract is observed in individuals who had suffered from serious infective bacterial illness and those treated with antibacterial drugs. A lower incidence was also observed in individuals who were not total abstainers from alcohol.

Some preliminary combinatorial analysis has shown the synergism of two risk factors, high levels of plasma urea and plasma fasting glucose and the varying counteractivity of high and low risk factors when combined.

The future role of epidemiological studies combined with biochemical studies to elucidate the mechanisms of cataractogenesis and to allow the identification of individuals who are particularly at risk with the aim of being able to take preventive measures is discussed.

CHAPTER ONE

THE STUDY OF THE LENS

1. Proteins of the lens

Protein accounts for approximately all the dry weight (20-40%) of the lens, (Harding and Dilley, 1976). Separation and classification of the proteins was initially carried out by Morner (1894) who used precipitation methods at various pH values. He designated the fractions α-crystallin, β-crystallin and albumin (γ-crystallin), plus the insoluble albumoid. This work was continued by Krause (1932) who obtained purer fractions of these components also by precipitation methods; α-crystallin precipitated at pH 5.2, β-crystallin at pH 7.2 and albumin (γ-crystallin) precipitated with saturated sodium chloride. Krause (1932, 1933) finding both chemical and physical differences between the four protein fractions suggested that they should keep the names given by Morner. He also differentiated the proteins by molecular weight calculated on the basis of amino acid composition; The minimal molecular weights for α-, β- and γ-crystallins he found to be 24,300, 15,100 and 24,000 respectively and suggested that real weights were integral multiples (73,000; 76,000 and 48,000 respectively) based on comparisons with serum globulin and serum albumin.

While the classification of Morner and Krause is still accepted, the complexity of these protein groups has only been elucidated within the last twenty five years during which time more sophisticated methods of separation and purification of the crystallins has allowed identification of a number of subunits within each group, more precise estimates of molecular weight and

and has also yielded information on the behaviour of these proteins within the lens in terms of ontogenic sequence, species differences and their role in maintaining transparency of the lens. Information on the crystallins from the whole of the vertebrate kingdom with regard to lens development is reviewed by Clayton (1974) and their role in ageing and cataractogenesis by Harding and Dilley (1976).

(i) α-crystallins

α-crystallin has the highest molecular weight of any lens water soluble protein and can be easily separated by various methods including gel filtration and ion-exchange chromatography. Early estimates of the molecular weight of bovine α-crystallins were varied; 12×10^6 (Francois, Rabaey and Wienne, 1955), 1×10^6 (Resnik, 1957) and 4×10^5 (Bon, 1959). Later work on bovine α-crystallin yielded figures of 8×10^5 (Bloemendal et al., 1962) and 1×10^6 (Spector and Katz, 1965) and further work by Spector et al., (1971a,b) indicated that the molecular weight of α-crystallin was not constant and they isolated three distinct macromolecular fractions in the weight ranges $6 \times 10^5 - 9 \times 10^5$, $0.9 \times 10^6 - 4 \times 10^6$ and greater than 10×10^6.

Bloemendal et al., (1962) showed that bovine α-crystallin was not a single macromolecule but that it consisted of subunits since the molecular weight decreased in dissociating conditions. These subunits were found to be non-identical but had similar molecular weights of around 25,000 (Schoenmakers and Bloemendal, 1968a) and would reassociate to form higher molecular weight complexes when dissociating conditions were removed (Bjork, 1964). Schoenmakers and Bloemendal (1968b), using isoelectric focusing, found 4 sub-

units with different isoelectric points (2 acidic and 2 basic) in adult bovine α-crystallin. These subunits were designated A_1, A_2, B_1, and B_2 (Waley, 1969). Spector et al., (1971) showed that the A-chains had a molecular weight of 19,500 and the B-chains, 22,500.

Thus α-crystallin was shown to be an oligomeric structural protein with around forty subunits per macromolecule. However, the subunit composition was shown to vary from the epithelium to the centre of the lens (Delcour and Papaconstantinou, 1970, 1974). One subunit (A_1) was found to be absent in the embryonic bovine lens and to increase with age (Schoenmakers and Bloemendal, 1968a; and Palmer and Papaconstantinou, 1969) and the subunits A_2 and B_2 were found to be predominant in the epithelium and young fibres where newly synthesised α-crystallin is present while the subunits A_1 and B_1 increased in amounts towards the centre of the lens, (Stauffer et al., 1974). A_1 was subsequently shown to be a post-translational product formed by a post-synthetic modification of A_2 chain possibly by deamidation of glutamine or asparagine (Palmer and Papaconstantinou, 1969; Bloemendal et al., 1972). More recent work (Delcour and Boucher, 1978) has suggested that B_1 may be formed from B_2 by a similar process. Van Kleef et al., (1974), who also found bovine α-crystallin in low, high and very high molecular weight aggregates, identified up to nine different polypeptide chains in aggregates from fibres of different ages. Van Kleef (1974) and Van Kleef et al., (1976) showed how these chains could be derived from previously existing α-crystallin by post-synthetic modifications such as deamidation and degradation. Stauffer et al., (1974) also found modified A-chains which had a reduced molecular weight in both calf and adult bovine lens.

While these investigations were carried out on bovine α-crystallin, further studies were carried out (Bjork, 1968) showing similar subunit composition and immunological homology between α-crystallin from different mammalian species. Later studies (de Jong et al., 1976) showed homology not only between mammalian species but between vertebrate classes although variations do occur in subunit composition and amino acid sequence. In each species, A_2 and B_2 are the only direct gene products, all other α-crystallin subunits arising due to post-translational modifications (Harding and Dilley, 1976).

As well as a changing balance in subunit composition between the nucleus and the periphery of the lens, a change in macromolecular size of α-crystallin has also been observed. In the bovine lens cortex, low molecular weight α-crystallin with a molecular weight of 0.7 - 1.7 x 10^6 predominates (Spector, Freund et al., 1971 ; Siezen & Hoenders, 1978) and towards the nucleus increasing amounts of the two higher molecular weight populations (1-10 x 10^6 and greater than 10 x 10^6) are found (Spector, Li et al., 1971b; van Kamp and Hoenders, 1973). Roy and Spector (1976) could find no low molecular weight species in the human lens nucleus and the high molecular weight aggregates contained peptides other than α-crystallin subunits. Aggregation of α-crystallin subunits will be discussed later.

(ii) β-Crystallin

While the nature of α-crystallin is reasonably well defined, the situation regarding β-crystallin is less so since it has not been so fully investigated. Separation of bovine lens crystallin

by gel filtration yielded two factions which varied in molecular weight (Testa et al., 1965; Francois et al, 1965; Zigler and Sidbury, 1973; Herbrink and Bloemendal, 1974) but which had similar amino acid composition (Bloemendal and Herbrink, 1974; Zigler and Sidbury, 1973) and immunological reaction (Bloemendal and Herbrink, 1974). Testa et al., (1965) estimated the molecular weights to lie in the ranges $1 - 1.5 \times 10^5$ (β_H) and $4 - 9 \times 10^4$ (β_L) while Zigler and Sidbury(1973) estimated them to be 2.1×10^5 and 5.2×10^4. The molecular weight of chick lens β-crystallin was found to range from 1.6×10^4 to 6×10^4 (Zwann, 1968; Truman et al., 1971).

β-crystallin was also found to be composed of subunits with an average weight of 25,000 (Bont et al., 1962) except for one native protein isolated by van Dam (1966) which had a molecular weight of 28,000 and did not dissociate. This was termed β_s-crystallin. Zigler and Sidbury (1973) found β_L to be a dimer consisting of two subunits of molecular weight 24,000 and 27,500 and β_H to be an oligomer of about eight subunits at least, one of which having a molecular weight 31,000 or 35,000, the others being either 24,000 or 27,000. Bloemendal and Herbrink (1974) found more subunits than the four found by Zigler and Sidbury but agree with them that some of the subunits are shared by each class although in different amounts. The subunit nature of chick β-crystallin was shown by Clayton and Truman (1967) who argued that β-crystallin was a family of heteropolymers, the subunits of which varied in number although the same subunits may be present in both the heavy and light fractions. Further work (Clayton, Campbell and Truman, 1968; Clayton, 1969; Truman et al., 1971;

Truman and Clayton, 1974) has confirmed this and shown that eleven subunits are present.

Immunological studies (Zigler and Sidbury, 1977) have shown cross-reactivity between β-crystallin of vertebrates of different evolutionary ages and indicate the conservative nature of these proteins. For such a heterogeneous population of subunits to arise, Clayton (1974) has suggested that a system of gene duplication and divergence has operated. However, it is probably that, as in α-crystallin, some subunits are not direct gene products but arise by post-translational modification of pre-existing subunits. In vitro studies of β-crystallin synthesis and aggregation (Vermorken et al., 1977) have suggested that the formation of β_H requires post-synthetic modification of at least one subunit which is present in calf lens nucleus but not in adult bovine lens nucleus. Thomson et al., (1978) and Zigler (1978) have also given evidence for post-synthetic modification of β-crystallin subunits in chick and bovine lens respectively.

(iii) γ-Crystallin

The "embryonic protein" of Francois and Rabaey (1957b) was shown by Bjork (1961) to be γ-crystallin and was found in larger amounts in young rather than old bovine lenses and in the nucleus rather than the cortex. This crystallin exists as monomers with a molecular weight around 20,000. Papaconstantinou (1965) could find no γ-crystallin in the epithelium and suggested that synthesis was induced during fibre formation during embryonic life. Bjork (1964b) purified bovine γ-crystallin by gel filtration and isolated from the preparation four distinct proteins differing in amino

acid composition, molecular weight and sulphydryl content although they have immunological identity thus suggesting that these are four distinct gene products. Slingsby and Croft (1972) divided one of these groups in two on the basis of difference in amino acid composition at the C-terminal end. Hines and Olive (1970) found eight components in rabbit γ-crystallin having similar amino acid composition to bovine γ-crystallin. Zigman et al., (1970) found eight components in rat γ-crystallin and later (Zigman et al., 1971b) found variations between species in the number of γ-crystallin constituents but found one common constituent with an isoelectric point at pH7.5. Differences observed between the individual γ-crystallins apart from amino-acid composition involve levels of sulphydryl groups and isoelectric points, and also the levels at which γ-chains are found in different parts of the lens. The higher the level of sulphydryl content, the higher the isoelectric point (Zigman et al., 1970) and those with higher sulphydryl content are found in higher concentrations in the older fibres in the nucleus while those with lower sulphydryl content are more abundant in the cortex (Zigman et al., 1971a). These authors suggest that the high level of sulphydryl groups in γ-crystallin is important in the formation of the insoluble lens protein fraction, the aluminoid[b]. Croft (1973), has suggested that γ-crystallin contributes to this process in humans where it had been previously identified immunologically in the albuminoid along with both α- and β-crystallins by Manski et al., (1968).

The loss of γ-crystallin from the soluble protein fraction of the lens with age and cataract has been observed in the human lens (Croft, 1973; Kabasawa et al., 1977a,b; Wagner and Fu, 1978),

although controversy remains as to whether the loss is due to leakage from the lens, first suggested by Francois and Rabaey, (1958), insolubilisation (Francois et al., 1965) or aggregation and incorporation into higher molecular weight species (Zigman et al., 1969; Harding, 1975).

(iv) HM-Crystallin

As well as the three main groups of crystallin in the water soluble fraction of the lens a high molecular weight component has been isolated. The definition of high molecular weight crystallin varies depending on the species from which it has been prepared. In the case of bovine lenses, HM-crystallin was first prepared by Spector, Li, et al., (1971) when they isolated three groups of α-crystallin which varied in molecular weight. The highest molecular weight class (1×10^7) was identical both in amino acid and subunit composition to the other classes and consisted solely of α-crystallin. In rabbit, the HM-crystallin fraction studied by Liem-The and Hoenders (1974a,b) consisted mainly of α-crystallin but did incoporate some β-crystallin. The study of human lens HM-crystallin revealed that the amino acid composition did not correspond to any one particular crystallin class (Jedziniak et al., 1973; Roy and Spector, 1976) although Maraini and Mangili (1973) did isolate large aggregates of α-crystallin with molecular weight above 1×10^7. The study of such a protein species was of interest since Benedek (1971) asserted that macromolecules of this size range at a certain concentration would disrupt the transparency of the lens. Therefore, could HM-crystallin be an intermediate step in the insolubilisation process of lens protein

associated with cataractogenesis?

An increase of the proportion of HM-crystallin in the lens was observed with ageing so that more was found in the older bovine (Spector et al., 1971b; van Kamp and Hoenders, 1973) rabbit (Liem-The et al.,1975a) and human (Jedziniak et al., 1975; Roy and Spector, 1976) nuclear lens fibres. Also Liem-The et al., (1975b) found that in the case of X-ray induced cataract in the rabbit, formation of the HM-crystallin was accelerated with a concurrent increase in the incorporation of β-crystallin. However, Maraini and Mangili (1973a) could find no difference in the levels of α-crystallin aggregates between cataractous and normal lenses.

Early studies on bovine α-crystallin aggregates showed that they could be dissociated in the presence of urea and, that, on the removal of the urea, the subunits would reaggregate to form macromolecules of a size similar to the starting material (Bloemendal et al., 1962). However, the degree of reaggregation was found to be incomplete both immunochemically (Bjork, 1964a) and in terms of chromatographic and electrophoretic properties (Spector et al., 1971). In the search for mechanisms of aggregation, various factors have been implicated in in vitro reaggregation studies including glucose (Spector et al., 1971a) calcium (Jedziniak et al., 1972) and some unidentified chromophores (Li, 1974). Recent work by Siezen et al., (1979) studying the interrelationship of monomeric, oligomeric and polymeric α-crystallin in calf lens nucleus has shown by electron microscopy how the globular units of monomeric α-crystallin form flexible chain like aggregates to form oligomers and that eventually three dimensional branched polymers are formed. They also found that the size of reassociated α-crystallin in vitro

was dependent on the concentration of degraded A-chains. Degraded polypeptides have also been implicated in human HM-crystallin (Roy and Spector, 1976$^{b}_{a}$).

(v) Albuminoid

Two components constitute the albuminoid; lens crystallins, which have become insoluble either through ageing (Fulhorst and Young, 1966) or by some cataractogenic agent, and lens fibre membrane material. The former component is, however, soluble in concentrated urea solution (8M) thus suggesting that it is formed by aggregation of the soluble crystallin due to disulphide linkages. It has been argued that this aggregation does not occur *in vivo* but is rather, an artefact brought about by the method of protein preparation viz. homogenisation in aerobic, non-reducing conditions, (Harding, 1972; Lasser and Balazs, 1972). Harding found increasing amounts of insoluble material with increasing coloration of cataracts even in anaerobic conditions but argued that his material was a result of covalent bonding other than disulphide bonds. However, the higher levels of insoluble material found with increasing colour must reflect the increasing susceptibility of proteins to aggregation with age and cataracto-genesis. Lasser and Balazs, 1972 describe a matrix of membrane components plus soluble protein which forms during homogenisation in air but which also forms during homogenisation under nitrogen which suggests that, to some extent, insolubilisation does occur in the lens. Mach (1963) showed a parallel increase in albuminoid and decrease in water soluble protein with age, especially in the nucleus. Manski et al., (1968), who also found this, investigated

the nature of albuminoid and found that all crystallin fractions α, β, and γ, were involved, the degree of the involvement varying with age (Manski and Martinez, 1971). Until then, it was widely accepted that only α-crystallin contributed to the formation of both bovine (Ruttenburg, 1965; Waley, 1965) and human albuminoid (Mehta and Maisel, 1968) although some workers had also implicated β-crystallin (Nordmann, 1949; Dische and Zil, 1951. York et al., (1971) looked for the synthesis of a pathological protein in the formation of albuminoid but could not detect it and later (York et al., 1972) found that human albuminoid shared immunological identity with the water soluble crystallins. The pattern in dogfish is that albuminoid is similar to γ-crystallin (Lerman et al., 1968; Mehta and Lerman, 1970) but this is probably a reflection of the fact that fish lens may contain up to 70% of its soluble protein as γ-crystallin with very little α-crystallin (Clayton, 1974). Similarly, in the rat, γ-crystallin plays an important role in the formation of aggregates which, in the cortex of the lens, constitute a soft gel-like matrix and in the nucleus, a firmer gel as nuclear sclerosis takes place.

Roy and Spector (1978a) isolated a number of polypeptides of varying size classes from 11,000 to 100,000 each of which showed a more complex composition, with respect to the number of component polypeptides, than would be expected from such apparently homogeneous fractions. A more detailed study of the 11,000 dalton fraction which is a significant component of insoluble protein (Roy and Spector, 1978b), showed it to be a 9,600 molecular weight class consisting of a number of similar sized polypeptides most of which have an amino acid sequence corresponding to tryptic digest products

of α-crystallin A-chains. Thus post-translational modifications of lens crystallins may be important in the formation of high molecular weight and water insoluble aggregates.

2. STRUCTURE OF THE LENS

(i) Development

At an early stage of embryogenesis, in the case of the human when the embryo is between 4mm and 9mm long, an induction between the mesoderm and ectoderm in the head region cause the formation of optic vesicles which grow out from the forebrain and which induce palisading of the overlying ectodermal cells to form lens placodes. The lens placode invaginates to form a lens cup which in turn is pinched off forming the lens vesicle. This is enveloped by the basement membrane (Primitive lens capsule) which had underlain the lens placode (Arey, 1975; Coulombre, 1965).

Cells at the posterior of the vesicle, proximal to the optic cup, elongate progressively to obliterate the lumen thus forming the primary lens fibres which no longer undergo mitosis. The anterior cells, however, remain as a cuboidal epithelial sheet which, while covering the mass of fibres, is continuous with it at the equator of the lens. Mitosis occurs throughout the epithelium initially but gradually declines in the central area and eventually ceases leaving around the equator of the lens a region of proliferation which is maintained. These dividing cells give rise to new elongating fibres which are deposited around the original fibre mass so that they meet both anteriorly and posteriorly compressing the original fibres which form the lens nucleus; the newly laid down cells forming the lens cortex whose growth is continuous, although gradually decreasing, throughout life.

(ii) Function

The function of the lens in the eye is refractory, focusing light onto the retina by way of its transparency and alteration of its shape and/or position (accommodation). Both these phenomena are associated with a balance of two parameters; a high concentration of protein to raise the refractive index and a high water content to make the lens softer and more deformable (Clayton, 1974). The two extremes of this situation are seen in fish, whose lenses must be refractive as possible since the corneal-water interface gives little refraction and which, therefore, have very hard lenses, with a high protein content, which must be moved along an axis relative to the retina to allow accommodation; and birds, who must accommodate very rapidly with high acuity and who, therefore, have lenses with a high water and low protein content. However, taking as an example the bovine lens as being between these extremes, the situation with respect to refraction has been described by Bettelheim and Wang (1974) who showed, by use of the minimum light scattering technique, that the refractive index of the lens is not constant but rather varies between the nucleus and cortex and that this variation is a gradual and symmetrical change in refractive index which is high in the nucleus and decreases radically out to the equator of the lens. This correlates with the protein concentration in the fibre cells.

(iii) Transparency

Refraction, of course, depends on transparency and various studies have been carried out to determine how the lens maintains its transparency. Trokel (1962) and Philipson (1973) both considered the uniform protein distribution within the lens fibres

and the absence of large fluctuations in refractive index to be the explanation. Benedek (1971) suggested that lens transparency is due to the wavelength of the Fourier components of the density fluctuations in the lens being less than half the wavelength of the transmitted light, and that opacities would develop as a result of light scattering due to long wavelength density fluctuations caused by an increase in the concentration of high molecular weight protein aggregates. Such aggregates have been found in the nucleus, as discussed previously, but not in the cortex, although cortical cataracts are caused by increased turbidity. Schachar and Solin (1975), using Raman spectroscopy, proposed a theory for lens transparancy based on the anti-parallel -pleated sheet arrangement of the lens proteins. They suggested that the proteins of the cortex are arranged in this manner for considerable distances, e.g. several thousand Angstroms. Therefore, because of the polar nature of these proteins, a slight tilting of one of them will cause tilting in the neighbouring protein right along the line but in decreasing magnitude. In this way a disorder opacity will be induced in the cortex. The authors suggest that this theory would account for the reversible instant opacification due to applied pressure in the lens, (Barber, 1974) rather than instantaneous water movement between the fibres which Barber proposes.

More recent work, however, in the study of light scattering and birefringence (the transmittance of a double or halved image) has elucidated some of the optical properties of the lens. The apparent optical isotropy of the lens is due to the balance of the two components of birefringence; form and intrinsic (Bettelheim, 1975). In the situation of the bovine lens, the arrangement of the

fibre cells gives rise to negative form birefringence; but this is balanced by the fibres themselves possessing intrinsic or orientation birefringence of the opposite sign. Therefore, optical anisotropy must exist within the cell and is brought about by the non-random arrangement of the macromolecular aggregates of the lens proteins. This situation would answer the question of why a number of distinct proteins are required by the lens (the α-, β-, and γ-crystallins) since different crystallins form special anisotropic aggregates that cancel the form birefringence and thus enhance transparency. Distortion of such an arrangement, e.g. by disorganisation of lens fibres or by swelling of the fibres either *in vitro* or *in vivo* by induced galactose cataract causes an increase in birefringence, i.e. reduction in transparancy, since an imbalance is created between form birefringence and intrinsic birefringence (Bettelheim, 1977).

Weale (1979) observed changes in birefringence in lenses under stress and made two predictions. Firstly, that women are more liable to cataract than men due to increasing lens thickness with age and, secondly, that myopes are at greater risk than hypermetropes because, in the former, the lens is subject to greater and more continuous stress. But he stresses that this physical phenomenon serves as a predisposing factor in the presence of other causes rather than as a sole cause of cataract.

CHAPTER TWO

THE STUDY OF CATARACT

1. History of the Study of Cataract

The most frequent and important lesion found in the lens is cataract i.e. opacity of the lens. The origin of the medical term cataract is unknown but is thought to stem from the latin "cataracta" and Greek "Kataraktes", both of which mean waterfall, suggesting perhaps that some comparison was made between the loss of transparency of the lens due to opacification and the whitening of water (normally transparent) due to the disturbance of its flow. The earliest diagnosis and surgery of cataract is undetermined although it is mentioned in early Egyptian and Indian writings from the second millenium B.C. The ancient Hindu teaching of Susŕuta was that cataract was a disease of the lens itself but later, in Greek learning, while the presence of the lens in the eye was recognised, opacification was thought of as being not lenticular, but due to accumulation and solidification of evil humours in the empty space (locus vacuus) between the lens and the pupil. This humoral theory which was accepted in Europe for sixteen centuries, was propounded by Celsus (c 25 B.C. - 50 A.D.), who described the crystalloid (lens) as being situated in front of the hyaloid and consisting of a drop of liquid (humour) resembling the white of an egg and constituting the site of the ability of vision. Under certain pathological conditions, the humour thickened and flowed into the locus vacuus where it solidified to form a suffusion or cataract.

Felix Platter, at the end of the sixteenth century, demonstrated that the lens was an optical medium and at the beginning of the following century, Fabricius and Aquapendente of Padua correctly described the position of the lens. Some surgeons now taught that cataract was an opacity of the lens itself but such dissenting opinion of the old humoural theory could not be published. Wemer Rolfinck, professor of anatomy at Jena, who dissected the bodies of executed criminals, demonstrated repeatedly that cataract was due to clouding of the lens but this view, was considered heretical until a French physician, Michel Pierre Brousseau, made the same observation on a dead soldier who had cataract and reported his findings to the "Academie Royale des Sciences" in Paris in 1705. Controversy then raged throughout Europe as opthalmologists such as Hermaan Boerhaave in Holland and Giovanni Battista Morgagni in Italy propounded the scientific fact that cataract was not an inspissated humour but a hardened and clouded lens. It was finally accepted when Jacques David published his classical paper on the deliberate extraction of cataract in 1753.

The history of the subject since then involves observations on morphology and concepts of aetiology i.e. the classification of cataract. It was the beginning of the nineteenth century, when some congenital cataracts were investigated, before any descriptions of morphology were made. The anterior polar cataract was described in 1817, the anterior capsular in 1887 and the posterior polar cataract in 1909. The advent of the slit-lamp microscope greatly facilitated detailed descriptions and the early observer Alfred Vogt of Zurich made accurate and painstaking observations

thus laying down the foundations of modern knowledge. There is as yet, no universally accepted classification of cataract but classifications do exist of chronology (congenital, neonatal, juvenile, presenile and senile), morphology (colour, cuneiform, cupuliform, coronary, etc.), topography (capsular, nuclear, cortical, polar, etc.) and development (incipient, intumescent, mature, hypermature). Pirie (1968) classified lenses on the basis of colour, ranging from yellow to black, dividing them into four categories, Groups I, II, III and IV.

Along with morphological observations, ideas on the aetiology of cataract were reported. Traumatic cataract due to contusion or perforation had been described in the sixteenth century but it was late in the nineteenth century when both heat and cold cataracts were noted. Radiation cataract due to roentgen rays was first reported at the beginning of this century and later by Miller, Fugino and Nafziger (1967) who observed them in survivors of the Hiroshima and Nagasaki bombings. Nutritional cataracts, were widely studied on an experimental basis and observed clinically in patients suffering from anorexia nervosa (Miller, 1958) and in inmates from a Japanese concentration camp (Ridley, 1920). A large number of toxic agents, including corticosteriods (Oglesby et al, 1961[a,b]), miotic drugs (Axelsson, 1973) and cytotoxins (Duke-Elder, 1972) and metabolic disorders including diabetes, (Duke-Elder, 1925; Caird et al., 1969) and galactosaemia (Kinoshita, 1965) were also implicated as cataractogenic agents.

2. Senile Cataract

According to Sorsby (1962), approximately 1.2 million people throughout the world develop visual impairment due to cataract annually, and Nordmann, (1972) states that lenses after the age of 60 are seldom free of opacities (Although to define them all as cataractous is debatable since not all opacities cause a decrease in visual acuity). McGuinness (1967) found a frequency of lens opacities in the general population of 48% at the age of 40 rising to 80% at 85. The three most common forms of cataract occurring at about 45 years of age or later are cortical (white soft cataract), nuclear (hard brown cataract) and cupuliform (posterior subcapsular saucer-shaped cataract.). Each may occur singly or be involved with one or both of the other types (Bellows and Bellows, 1975). A fourth type of opacity found in almost all adult lenses, is described as punctate. They do not usually cause visual impairment except where, with increasing age, their numbers increase, or where there is a second type of opacity involved.

3. Factors Contributing to Senile Cataractogenesis

While cataract is regarded as a sign of old age, other factors may be implicated. Senile cortical cataract has been ascribed to causes such as endocrine disturbances, nutritional deficiencies, toxins, radiation, alterations in the lens capsule, osmotic imbalance and hereditary factors, (Bellows and Bellows, 1975; Duke-Elder, 1969). Hockwin, (1976) quotes Becker, (1877); "the cataracta senilis is, to a certain degree, a normal symptom of age, a kind of physiologic lens death, and, as such, unavoidable. However, the fact that the time of manifestation is different with each individual indicates the supervention of a casual cause".

Also the diversity of the nature of cataract, both in morphology and in the number of cataractogenic agents, makes it necessary to consider several factors, either singly or cumulatively, in the aetiology of the disease. Cataracts which present clinically include congenital, senile, metabolic (e.g. diabetic, galactosaemic, myotonic, aciduria, etc.), osmotic, toxic and traumatic cataracts plus those which appear as part of some multiple syndrome. Some of these cataracts, which may involve aggregation of lenticular proteins, abnormal ionic exchange, osmotic changes within the lens which disrupts metabolism or injury to the lens cells, can be imitated by experimental induction. Methods of producing cataract include physical causes (such as osmotic influences, cold and heat), radiation of varying wavelengths, interference with nutrient supplies either by addition or depletion of certain metabolites, and induction of sugar cataracts (by feeding with galactose, xylose or glucose) and toxic cataracts (by addition to the diet of naphthalene, paradichlorobenzene, enzyme inhibitions and other cataractogenic drugs), (Duke-Elder, 1969).

Much research has been done to investigate the process of cataractogenesis using human lenses after surgery or animal lenses in which cataracts, have been experimentally induced, (Nordmann, 1972; Barber, 1973; Harding and Dilley, 1976). Hockwin and Koch (1975) discuss the mechanisms of cataract formation and the value of experimental investigations in the understanding of the aetiology of human cataract. In experimentally induced cataract, such as with galactose (Kinoshita, 1965; Kinoshita et al., 1963) and naphthalene (van Heyningen and Pirie, 1967; van Heyningen, 1979), the biochemical and morphological changes have been described in

detail. But in clinical cataract, it is difficult to define one mechanism as being the cause, since some cataractogenic agents while producing an identifiable characteristic cataract in some cases, will in others, induce earlier onset of what is commonly described as"senile cataract". Also, more than one type of opacity or cataractogenic factor may be involved in the formation of cataract within one lens; this is what Hockwin and Koch (1975) term a "multifactorial" cataract.

However, in the following sections, nuclear, cortical and posterior subcapsular cataracts will be considered individually in terms of pathology, morphology and, where the data is available, biochemistry. Much information is available on the subject of brown nuclear cataract and is included within that section. Some cortical cataracts, including some subcapsular cataracts, whose aetiology is fairly well understood are discussed individually after a general description of cortical cataract.

4. Brown Nuclear Cataract

(i) Lens Colour and Fluorescence

The lenses of many nocturnally active animals such as rats, rabbits, guinea pigs and most fishes, are colourless while in most diurnally active animals, for example geckos, snakes, tree shrews, squirrels, monkeys and man, the lenses are various shades of yellow, amber and brown, these colours developing with age (Zigman, 1971; van Heyningen, 1976). These authors suggest that this colouration acts as a filter against ultra violet light so that the retina is protected against such irradiation which might otherwise depress synthesis of proteins and RNA in that tissue. While van Heyningen

states that filters are present in the lenses of some species other than man and the primate, the argument for such an evolutionary adaptation requires a comprehensive study of vertebrate lens before it could be acceptable. It has been proposed that the formation of the yellow colour, while acting as a U.V. light filter, may in fact be caused by such irradiation in the first place and that prolonged exposure might lead to extensive colouration and the formation of a nuclear cataract (Pirie, 1972).

Nuclear cataract is said to be present when physiologic sclerosis exceeds its normal bounds and is characterised by the dark colour and unusual size of the nucleus and the presence of dustlike opacities in the nuclear region (Bellows and Bellows, 1975). Nordmann (1972) differentiates two groups of nuclear cataract; the typical nuclear cataract and the brunescent cataract. Development of the forms is similar and involves both pigmentation and sclerosis of the lens nucleus but in the brunescent cataract the cortex may also become involved so that the supranuclear layers become coloured. Since cortical involvement occurs at a later stage of cataractogenesis, the pigmentation there is not so intense as in the nucleus and a range of colours from black through brown and amber to yellow may be observed in some cases from the nucleus outwards and such an observation led Vogt (1931) to suggest that the darker colours were just an intensification of the yellow pigmentation. However, other workers (Said and Weale, 1959; Cooper and Robson, 1969) have suggested that several coloured substances, which may give rise to different levels of pigmentation, exist in the lens. Zigman (1971) identifies these substances as aromatic compounds including the amino acids phenylalanine, tryptophan and tyrosine

which, as a result of photo-oxidation of the aromatic rings to quinones, become yellow, brown or black respectively.

Associated with the increasing brown colouration of human lenses, whether normal or cataractous, is an increase in fluorescence at wavelenghts longer than 400 nm. (Lerman, 1972, 1976). Satoh (1973) describes two types of fluorescence; purple, which he attributes to the tryptophan and tyrosine residues in the protein matrix, and blue, the nature of which chromophore is unidentified. The latter, he claims, is responsible for the increased fluorescence in age and cataract. Lerman and Buckman (1976) also found two fluorogens one of which they suggest is responsible for the normal yellow colour in ageing lenses and the other for the deeper yellow to brown colour observed in advanced nuclear cataracts. Earlier work by Francois, Rabaey and Recoules (1961) revealed a substance of low molecular weight which gave a blue-green fluorescence and which could only be prepared from human and primate lenses (which, according to van Heyningen (1976) are the only lenses which develop brown nuclear senile cataract). This low molecular weight substance was thought most probably to be a peptide since the absorbance peak at 270 nm. obtained in fluorescence studies might be explained by the cyclic amino acids such as tyrosine, tryptophan or phenylalanine (the amino acids susceptible to photo-oxidation) (Zigman, 1971).

(ii) Protein Changes in Brown Nuclear Cataract

(a) Photo-oxidative Changes

In order to investigate the effect of photo-oxidation, Pirie (1972) exposed lens proteins and tryptophan to sunlight and observed the following changes, which did not occur in control conditions in the dark; the protein solution turned yellow and golden brown while tryptophan in aqueous solution turned a deep brown with, in some cases, a deposition of a black precipitate. The lens proteins tended to become insoluble and fluorescent studies showed them to be similar to protein prepared from brunescent cataract in that both showed decreased levels of histidine and tryptophan. Increased fluorescence has been associated with insolubilisation of protein due to covalent non-disulphide bonds which appear in the final stages of brunescent cataract (Dilley and Pirie, 1974; Bando et al., 1976). However, Zigler (1976) could find no difference between the amino acid composition of normal cataractous lenses except that free tryptophan in the amino acid pool was reduced in brunescent cataract. Earlier studies (Pirie, 1971) had revealed that tryptophan is oxidised through the action of sunlight to give N-formylkynurenine and Zigler et al., (1976) found traces of kynurenine in brunescent cataract. Kurzel et al., (1973) and Yamanashi and Zuclich (1978) also implicated photo-oxidative products of tryptophan in lens pathology. Kynurenine could act as a photosensitiser or react directly with the proteins of the lens. Since the nuclear proteins of the lens are particularly rich in tryptophan and are also long lived, early oxidative changes may lead over a period of many years to cataractogenesis and the formation of brown nuclear cataract.

(b) Formation of Disulphide Bonds

Truscott and Augusteyn (1977b) could find no change in tryptophan levels in cataractous lenses but did observe changes in the levels of methionine and cysteine both in nucleus and, to a lesser degree, in the cortex in later stages of cataractogenesis. They also found, as did Testa et al., (1968) and Auricchio and Testa, (1972), increased levels of disulphide and decreased levels of sulphydryl groups. They argued that photo-oxidation was not the cause, since light of the appropriate wavelengths was not transmitted by the cornea, but rather that oxidation was due to oxygen or peroxide. This argument was linked with the levels of the free radical scavenger glutathione which drop in the nucleus in brown cataract but remain constant in the cortex (Augusteyn, 1979). Two theories have been forwarded to explain the formation of disulphide bands. Truscott and Augusteyn (1977c) and Rogers and Augusteyn (1978) have suggested that since glutathione reductase controls the levels of reduced glutathione in the lens, changes in brown nuclear cataract may, in part, be the result of a defective enzyme. Alternatively, the susceptibility to oxidation of the thiol groups within the protein may be enhanced by conformational changes which the proteins may undergo during cataract development (Kinoshita and Merola, 1973; Harding, 1972, Auricchio and Testa, 1973). Such conformational changes would expose the thiol groups and promote the formation of intermolecular disulphide bonds.

(c) Protein Insolubilisation

Measurements on the degree of insolubilisation of nuclear protein during cataract development have been made by a number of workers. Dilley and Pirie (1974), and Buckingham (1971, 1972) in comparing Group IV cataracts with other lenses both normal and paler yellow, found a high proportion of urea insoluble protein in the nucleus of the former, while in the cortex, the ratio of 25mg urea soluble to 4mg of urea insoluble was the same as in the cortex of other lenses. Kramps et al., (1976) and van Haard et al., (1978) also found the ratio of water soluble to urea soluble to urea insoluble within the cortex of both normal and cataractous lenses to be the same while quantitative changes had occurred in the nucleus during cataractogenesis. A decrease in the water and urea soluble fractions with a concomitant rise in the urea insoluble fraction was observed without any change in dry weight which suggested that these changes are mutual. Since the addition of a reducing agent results in the solubilisation of the urea insoluble fraction (Kramps et al., 1976; Truscott and Augusteyn, 1977a), insolubility must be due mainly to disulphide cross links. Truscott and Augusteyn, (1977a) proposed a lattice network of protein subunits joined by disulphide bands with other covalent bonds also involved since increasing colour was paralleled by an increased inability to be solubilised in the presence of a reducing agent.

(iii) A Question of Location

Senile brown nuclear cataract is associated, as has been described, with the accumulation of coloured insoluble proteins which are localised in the nucleus of the lens. While this is undisputed, the cause and mechanism of the formation of such

aggregates and the reason why such changes should be restricted to the nucleus remain hypothetical. Why should the nucleus only be liable to pigmentation if, as Pirie (1971) and Zigman (1971) have shown, all the proteins from the lens will undergo the changes described if exposed to U.V. light? The role of ascorbic acid in the prevention of photo-oxidation of proteins within the cortex has been suggested as one possible factor (Pirie, 1972). Truscott and Augusteyn (1977b) did not accept photo-oxidation as a cause but preferred free radical oxidation of sulphydryl groups. But this too begs the question as to why only the nucleus should be involved until the very advanced stages of cataractogenesis. The possibility of control of disulphide band formation by either glutathione reductase or conformational changes within the proteins leads to the same question. One factor which is significant in this apparent paradox is the difference in protein composition between the cortex and the nucleus. Within the nucleus are proteins which undergo little or no turnover throughout life, are rich in tryptophan and sulphydryl groups and throughout life are exposed to influences, such as sunlight and other radiation, which induce cross-linking and insolubilisation (Maraini and Mangili, 1973).

A number of weaknesses are present in all these arguments put forward. First of all, lenses are classified post-operatively and therefore lack a detailed description of their cataractous morphology as provided by careful slit-lamp examination <u>in situ</u>. Secondly, lenses are selected or discarded on the basis of morphology and then pooled before analysis so that a great deal of information is probably lost and only generalised statements can be made from the results. Finally, it seems that all these workers assume that

a brown cataract is a progressive phenomenon, i.e. it begins by being yellow and gradually deepens in colour due to intensification of the yellow pigment, and therefore they have all looked for progressive changes biochemically. But if, as suggested by Said and Weale (1959) and Cooper and Robson (1969), different levels of pigmentation are due to different coloured substances, then the premise of progression is a wrong one.

5. Senile Cortical Cataract

Senile cortical cataract is characterised mainly by degener - ative processes in the cortical fibres, their membranes and in the epithelium which result in the formation of water clefts, cuneiform opacification and lamellar separation, (Nordmann, 1972; Duke-Elder, 1969). The stages of cortical cataract which can be clinically observed are as follows:- incipient or immature, intumescent, mature, hypermature and Morgagnian. The initial stages have been studied by electron micrscopy, (Philipson, 1973; Philipson and Fagerholm, 1973) and are characterised by an enlargement of extracellular spaces, disruption of lens fibres and breakdown of cell membranes. These phenomena depend on the imbibition of water by the lens following breakdown of the cortical fibres and the consequent increase in osmotic pressure. As the amount of free water in the lens increases, the size of the lens increases with a concomitant increase in the number of vacuoles and clefts as fluid moves between the individual fibres. This represents the intumescent stage. Subsequently, the disintegrated products may excape by leakage through the capsule so that the lens decreases in size until the cataract reaches maturity and, later on,

hypermaturity when the lens becomes shrunken and yellowish with irregular deposition of grayish-white material in the cortex. If, however, the capsule is abnormally thick (Fukui and Yamashina, 1978), so that leakage does not occur, the lens appears as a bag containing a milky white fluid within which the nucleus has sunk due to gravity - the Morgagnian cataract.

6. Posterior Subcapsular (Cupuliform) Cataract

Cupuliform cataract is a common form of senile opacification consisting of a thin layer of granules located just beneath the capsule in a "saucer shape" and in slit lamp microscopy appears yellow, (Duke-Elder, 1969). This form of cataract occurs at an earlier age than nuclear or cortical cataract, progresses slowly and often becomes complicated with other opacities. Because of its location, visual function is impaired at an early stage (Bellows and Bellows, 1975; Duke Elder, 1969).

Streeten and Eslaghian (1978) studied fifteen human lenses with posterior subcapsular cataracts histologically and compared them with normal and non-posterior subcapsular lenses. The shape of the cataract was either polygonal or stellate, correlating to some extent with the suture pattern, and a semiliquefied zone was present in the centre. Cells migrating from the equatorial zone were observed and a disorganisation of the postequatorial nuclear rows was more pronounced than in control lenses. The migrating cells were stellate in shape and in more advanced cataracts were oriented in a ring round the semiliquefied zone where bladder cells and other proliferative cells tended to accumulate. The morphology described here was very similar to that in posterior subcapsular

cataracts induced experimentally by Cogan, Donaldson and Reese (1952) using radiation, and in those observed by Greine and Chylack (1979) who studied posterior subcapsular cataracts produced by long term steroid therapy. Creighton et al., (1978) studied posterior subcapsular cataract by scanning electron microscope and found globular bodies present in the cataractous area. They suggested that the globules were formed during fibre degeneration when sub-surface actin microfilaments fragment and form Morgagnian globules when the cell forms blebs at the surface. But no factor is known which will stimulate this process and so cause cataract in the posterior cortical area.

In the study of posterior subcapsular cataracts produced experimentally by tryptophan deficiency by Ohrloff et al., (1978), the authors suggest that, in the light of evidence given in that paper and supported by the results of Koch (1976) and Koch et al., (1977), this type of opacity may be due to disrupted protein metabolism. This theory is also supported by the observations of McAvoy and van Heyningen (1976) who showed that terminal differentiation of fibre cells was abnormal in terms of cell growth and denucleation. Friedburg, Kroner and Rosentiel (1974) suggested that disrupted protein metabolism may be the reason for the development of glucocorticosteroid induced posterior subcapsular cataract. These authors observed inhibitions of protein synthesis, indicated by decreased incorporation of leucine-1-(^{14}C), in lenses treated with glucocorticosteroid as opposed to the mineral corticosteroid aldosterone, which produced no such decrease.

7. Metabolism and Cataract

Metabolic dysfunction, however induced, is a major cause in cataractogenesis. Metabolism is essentially a three step process - supply of nutrients, conversion and synthesis, and expulsion of waste products (Koch, Hockwin and Ohrloff, 1976), - and will be disturbed if any one step goes beyond the regulated limits, e.g. lack or excess of nutrients, enzymic block or hindered elimination of end products. A disturbed metabolism will lead eventually to changes in either or both the water content of the lens and the protein conformation within the lens due to changes in osmotic pressure, a common feature of many cataracts (Kinoshita, 1974).

Different metabolic processes may be involved in different forms of cataract, e.g. carbohydrate metabolism in diabetic and sugar cataracts, lipid metabolism in triparanol and some other drug induced cataracts and protein synthesis in deficiency and steroid cataract. Metabolism may also be disrupted by the effect of a toxic substance within the lens, as in the case of napthalene cataract, or by some external agency, as in the case of radiation cataract. Some of these cataract types will be discussed more fully.

(i) Diabetic and Sugar Cataract

(a) Epidemiology

The incidence of cataract in association with Diabetes mellitus was first observed by Berndt in 1834 and by many others since. Bellows (1944) cites a number of cases where cataract had been diagnosed in diabetic patients but only a small percentage (1-2%) were true cataracta diabetica (see following section), the rest being due to the early onset of senile cataract. True

diabetic cataract is more frequent in children and may be observed in up to 20% of patients (O'Brien et al., 1934). Opacities usually occur during adolescence, although Major and Curran (1925) recorded a case in an 11 month old infant and Lawrence (1946) found transient[5] opacities in older patients with poorly controlled diabetes. Bellows considers 40 years as the age limit beyond which true diabetic cataract cannot be confidently diagnosed, but other authors have set a lower limit; Gray (1933) at 36 years and O'Brien et al., (1934) at 33 years. In older patients, statistical analysis is necessary to determine whether cataract may have some diabetic origin. Gallus (1919) and Poulard (1923) denied that the diabetic state might contribute to cataract formation and Kirby and Weiner (1933) maintained that tendency to diabetes, as indicated by decreased sugar tolerance, and cataract are both manifestations of the ageing process but without any cause and effect relationship. Romer (1912) thought that diabetes might lead to an identifiable characteristic cataract at any age, but many opthalmologists including Shepherdson and Crawford (1931), Duke-Elder (1925) and Kirwan (1933) believed in the aggravation by diabetes of opacification of the lens initiated by senility. Kirwan (1933) observed the increased incidence of early senile cataract in association with diabetes in Bengal where there is a high incidence of diabetes developing in early middle life and Anthonisen (1936) showed that in every age group from 25 to 75 years, the incidence of cataract in diabetic patients was greater than would be anticipated from the statistics for the general population. Chaikoff and Lachmann (1933) carried out an experiment where 8 out of 10 pancreatectomised dogs developed cataract within

two years while no cataract developed in a large number of control animals. The importance of poor diabetic control in cataractogenesis in diabetic dogs was also demonstrated by Ricketts et al., (1959) and in alloxan diabetic rats by Patterson (1951). Patterson (1952) also related cataract formation to the severity of hyperglycaemia; rats with high blood-sugar values developed cataract in 8 to 10 weeks bilaterally while those rats with low blood-sugar values developed cataract more slowly with the lesion sometimes being unilateral. Thus, in the thirties there was much controversy concerning the cause and effect in this form of cataract, but some statistics, which were subsequently demonstrated and accepted, showed that cataract extraction is more common in diabetics (Caird, Hutchinson and Pirie, 1964; and Burditt and Caird, 1968) and that diabetics requiring cataract extraction have poorer control of diabetes than the average diabetic without cataract (Caird, Hutchinson and Pirie, 1964).

Recent studies (Irvine, 1977), have indicated that the previous classification of iodiopathic diabetes mellitus as juvenile-onset and maturity-onset types or insulin-dependent and insulin-independent types is unsatisfactory since insulin dependence or independence may be present in both juvenile and maturity-onset types of the disease. Autoimmmunity and histocompatibility studies, have, however, differentiated two types of the disease based on the presence or absence of pancreatic-islet cell antibodies (I.C.A.) in the serum correlating with prevalence or non-prevalence of the HLA marker, B8; presence of I.C.A. and increased prevalence of B8 indicating insulin dependence and absence of I.C.A. with no particular prevalence of any HLA phenotype indicating insulin

independence (type II). While these differences are irrespective of age, type I tends to occur under the age of 35 while type II tends to occur above that age. This would account for the differences in opinion during the thirties regarding the dividing line above which the true diabetic cataract could not be diagnosed with certainty. A survey of the incidence of cataract in the two types of the disease as described by Irvine (1977), would be useful in determining whether the HLA system is important in the aetiology of cataract. That this is a possibility is predicted by the role played by membranes in the formation of certain cataracts, especially those involving vacuolarisation and the formation of clefts due to imbibition of water and the separation of fibre cells which are usually tightly packed and whose membranes are highly interdigitated.

(b) Pathology

The morphology of diabetic cataracts was described by O'Brien and Allen (1942) and they differentiated between two forms:-

1. The "true" diabetic cataract, characteristic of the disease.
2. A form similar to senile cataract but with early onset and maturing more rapidly.

The true diabetic cataract occurs primarily in juveniles, is bilateral, simultaneous and shows rapid onset and early maturity. Opacities consist of blue or white punctate opacities scattered in the anterior and posterior subcapsular areas. The dots often become confluent in the posterior subcapsular area giving a saucer shaped opacity, granular in nature and perhaps containing irridescent crystals. As the cataract develops, vacuolisation of the entire subcapsular area occurs and clefts may form between the

lenticular fibres. The second type of opacity involves sclerosis of the lens nucleus in adult diabetics and they have been found to be similar to non-diabetic senile cataracts both histologically (Gordon, 1949) and biochemically (Carey and Hunt, 1935) although more recent studies have elucidated the biochemical situation (van Heyningen, 1959 and 1962; Kinoshita et al., 1962a,b; Kinoshita, 1963).

The pathogenesis of cataract formation in diabetes mellitus is still not fully understood but evidence suggests that altered osmotic relationships may be the underlying cause. Duke-Elder (1925) described cataractogenesis in diabetics as being due to the denaturing action of radiant energy in the presence of excess sugar and acetone. If the sugar concentration was high then the salt concentration must drop to maintain the osmotic balance, but since sugar transport is slower than salt transport, the lens will take up water from the aqueous which will have a lowered osmotic pressure. This causes swelling of the lens and myopia and if hydration is very severe, vacuoles will form under the capsule, the lamellae will separate and transparency will be impaired. Van Heyningen (1959) first studied the carbohydrate metabolism in the rat lens where cataract had been induced by high levels of xylose and galactose and by alloxan induced diabetes. She found in the lens, high levels of xylitol, dulcitol and sorbitol respectively, thus demonstrating the presence of an aldose reductase enzyme and assumed the reduction of glucose to sorbitol as a normal metabolic process. Kinoshita et al., (1962a,b) also demonstrated the accumulation of dulcitol in the lens of galactose fed rats and suggested that the hypertonic condition thus created

would lead to a net increase in water content sufficient to cause rupture of the lens fibres. This would explain the histopathological changes occurring in the initial phase of experimental galactose cataract. The enzymatic process which accounts for the production of sorbitol when glucose levels are raised is explained by van Heyningen (1962) and Kinoshita (1963). They both demonstrate that hexokinase, the enzyme which phosphorylates glucose in the first stage of glycolysis, has a higher affinity for glucose than aldose reductase, but as glucose levels are raised, the hexokinase becomes saturated and excess glucose is available to be converted.

An alternative to insulin therapy has been suggested by Chylack and Kinoshita (1969) and Gabbay and Kinoshita (1972) who demonstrated the inhibition of aldose reductase by various chemicals thus preventing the accumulation of sorbitol and delaying experimental cataract. Various flavanoids were tested in vitro (Varma and Kinoshita, 1976) and quercetin proved effective at low concentrations (10^{-5}M). Topical application of quercetin to galactosaemic neonatal rats (Beyer-Mears and Farnsworth, 1979) was found to reduce fluid accumulation and maintain interdigitation of the cortical fibres without affecting the lens growth rate.

(ii) Miotic Induced Cataract

Glucose metabolism has also been shown to be disturbed by the cholinesterase inhibitor drug, demacarium (Michon and Kinoshita, 1968a,b). This is one of a number of miotic drugs used to treat glaucoma and causes increased lactate production and increased oxygen consumption. Other miotic drugs which have been associated with the production of anterior and posterior subcapsular

vacuolarisation and cataract include disopropyl fluorophosphate, paraoxon and echothiophate iodide. As well as disturbed glucose metabolism, Michon and Kinoshita (1968a,b) found increased amounts of sodium and water and decreased amounts of potassium in treated lenses and suggested that the primary lesion caused by these drugs is increased lens permeability since changes in sodium and potassium levels are observed even in a hyperosmotic environment where the lens is prevented from swelling.

Philipson et al., (1979) studied the subcapsular cataracts induced by echothioplate iodide in monkey lenses. Light and electron microscopy revealed radial patterns of vacuoles and enlarged intercellular spaces containing membrane fragments and other amorphous material which was possibly protein. These authors make no suggestion as to a possible mechanism but the observations indicate some possible effect on membrane synthesis which would account for the increased permeability suggested by Michon and Kinoshita (1968b), and the subcapsular site of the lesion where there is a high rate of membrane synthesis.

(iii) Triparanol Cataract

Vacuolarisation causing anterior and posterior subcapsular opacities due to triparanol, a cholesterol reducing drug, has been observed clinically, (Kirby, Acher, Perry and Winklemann, 1962), and has also been induced experimentally in rats (von Sallmann, Grimes and Collins, 1963). Recent work by Harris and Gruber (1969, 1972, 1973), Rathbun, Harris, Vagstad and Gruber (1973) and Rathbun, Hough, Gruber and Harris (1978), has shown triparanol cataract in rats to be totally reversible and that factors involved such as increases in sodium and water content and decreases in total protein,

(characteristics of most cortical cataract), return to normal as the cataract clears. In studying metabolism with this system, these authors made a number of observations; only one of two sodium pumps present in the lens was affected, (Harris and Gruber, 1972), transport of α-aminoisobutyric acid was decreased, (Harris and Gruber, 1973) and glutathione levels remained unaffected (Rathbun, Harris, Vagstad and Gruber, 1973). These findings, since they involve membrane properties, and since triparanol affests lipid metabolism suggest that cataractogenesis is due to some disturbance in this metabolism as originally suggested by von Sallmann, Grimes and Collins, (1963) and Mizuno et al., (1974).

Anterior and posterior subcapsular opacities were also induced in rats by the amphiphilic drugs iprindole (an antidepressant) and Chloroquine (an antimalarial and antirheumatic drug) which affect lipid metabolism (Drenckhahn and Lullmann-Rauch, 1977). Rats treated with non-lipidosis-inducing analogues showed no sign of cataract. The subcapsular regions are most susceptible, say the authors, since they have a high lipid turnover and inhibition of lipid metabolism will cause a lack of molecular residues required for membrane synthesis.

(iv) Dietary Deficiency Cataract

An example of cataract due to dietary deficiency is that caused by a lack of the essential amino acid tryptophan (Koch, Hockwin and Ohrloff, 1976). This opacity, a posterior subcapsular cataract, has been produced experimentally in rats (Buschke, 1943) and guinea pigs (von Sallmann, Reid, Grimes and Collins 1959). Buschke (1943) describes two types of opacity; acute, where feathery opacities occur in the posterior cortex followed by

perinuclear and nuclear opacification so that the whole lens becomes opaque in a short time, and chronic, where dot-like opacities occur subcapsularly with the rest of the cortex and nucleus remaining transparent. Histological studies (McAvoy and van Heyningen, 1976) have shown that tryptophan deficiency interferes with terminal differentiation of the fibre cells so that nuclei do not disappear as early as in normal lenses thus affecting the structure and transparency of the cortical fibres.

The authors suggest that while DNA breakdown is disrupted, RNA breakdown is not. If this is the case and it is combined with reduced protein synthesis due to amino acid deficiency, then it is likely that DNAase enzymes will be affected more so that denucleation will depend on the levels of DNAase already present which will continue to decrease if no synthesis is being carried on. Lack of an essential amino acid must, by definition, affect protein synthesis. While earlier workers employed decreased levels of tryptophan in the diet (van Heyningen, 1976), Ohrloff et al., (1978) decided to feed rats on a tryptophan-free diet. Like van Heyningen (1976), they observed a slower growth rate of the lens during treatment in that both the wet and dry weights were lower than normal suggesting that protein synthesis is decreased. When the protein contents of the posterior and anterior portion of the lens were studied, (Ohrloff et al., 1978), no difference could be found in the extent of decreased synthesis between them, although opacification occurs only posteriorly. They also suggested that all crystallin α, β- and γ-, were affected, though Dische et al., (1959) found a decrease mainly in the β-crystallins. But the comparison is not justified since Ohrloff et al., analysed proteins

from the anterior and posterior subcapsular regions only and not from the whole lens as Dische had done. Also, Dische used only a decreased tryptophan content in the diet while Ohrloff et al., used a tryptophan-free diet. Thus, in the case of the latter, all proteins requiring tryptophan and being synthesised during the course of the experiment will be affected while in the presence of small amounts of tryptophan, there may be some preferential effect if crystallins are synthesised at different rates or at different times. Ohrloff et al.,(1978) suggest that protein synthesis is not permanently damaged since the cataract is reversible when tryptophan is reintroduced to the diet. This is not a logical conclusion from this data since what they observe is a resumption of normal metabolism in fibres being laid down post hoc while the opaque fibres become compressed but remain opaque and form a cuplike granular cataract separated from the subcapsular regions and presumably unaffected by the higher levels of tryptophan which may not be transported to the inner regions of the lens.

(v) Steroid Cataract

Posterior subcapsular cataracts (PSC) are generally associated with exposure to toxic agents, posterior intraocular disease, ionising radiation or blunt trauma. Black et al., (1960) were the first to notice PSC in four rheumatoid arthritis patientswho had not been exposed to any of the above aetiological factors but had received prolonged treatment with synthetic corticosteroids. From a survey of forty seven patients receiving corticosteroid therapy and nineteen control patients (66 rheumatoid arthritis), 17 treated patients (39%) and no controls developed PSC. No correlation could be made with the degree or duration of the disease

while dosage and duration of corticosteroid therapy correlated markedly. Studies by Giles et al.,(1962) supported their findings - a 37% incidence of PSC in patients treated with corticosteroids, the dosage and duration of therapy being the major determining factor in the formation of the cataract. Williamson et al., (1969), in response to the observations made by the above authors plus others (Oglesby et al., 1961; Crews, 1963; Spencer and Andelsson, 1965) who found a relationship between corticosteroid therapy and the incidence of PSC with a range of 12.5 to 60%, carried out a survey of 365 patients, approximately half of whom had received long term therapy of systemic corticosteroids. They found an incidence of PSC in 6% of the treated and 0.6% of the non-treated patients with a relationship between the degree of opacity and the dose and duration of therapy. Another study by Kern, Zaruba and Scheittin (1970) on the ocular side effects of immunosuppressive therapy in kidney transplant patients also showed an increased incidence of cataract of the type associated with steroid therapy and dependent on the dose and duration of therapy. The subcapsular cataract occurring in 13 out of 28 patients whose average age was approximately 31 years (the average incidence of spontaneous cataract at this age is 0.6%)could be differentiated from a second type of punctate opacity which was due to renal dysfunction and occurred in five of the patients.

The question raised by these observations is that there is an increased incidence of cataract in patients treated with corticosteroids and while the morphology of the cataract is characteristic, why does steroid not induce cataract in everybody? Is some other cataractogenic agent involved and if so unless only one agent

is involved, why is the morphology consistent? Or is there some inherent genetic influence which predisposes liability on a certain percentage of the population? To answer these questions, a study of experimentally induced cataract is required where the necessary controls can be included. However, the association of drug and lens opacification which could be observed in humans, could not be produced experimentally in rats, rabbits or chickens (Bettman et al., 1968). Steroid cataract has been induced in guinea pigs, however, by Friedburg, Kroner and Rosentiel (1974) who also observed that, while glucocorticoids would produce a specific effect, mineralocorticoids (aldosterone in this case) did not produce cataracts. Their studies on the biochemistry showed that intravitreal injection of methylprednisolene caused a transient increase in sodium concentration associated with the appearance and disappearance of subcapsular vacuoles. Transient vacuolarisation is probably due to water imbibition during the time of increased sodium concentration or alternatively to injection trauma which is gradually overcome. What they consider as more important in the mechanism of steroid cataract is the decreased (^{14}C) leucine incorporation which they observe, as being more permanent than the cation level changes. If steroids do inhibit protein synthesis then the effect over a long period of therapy would be expected to be cumulative and have severe consequences.

A series of studies by Ono, Hirano and Obara (1972 a,b,c and 1973) elucidates the role of cortisol metabolism within the lens in cataractogenesis. They found that cortisol in the lens underwent sulphate and glucomide conjugation or became bound to proteins, β-crystallin in particular. Species differences were found in

conjugation activity between human, bovine, rabbit and rat, human lenses having the highest activity and the authors suggested that this was related to interspecies differences in steroid hormone metabolism. In comparisons between normal and cataractous human lenses, a decrease was observed in sulphate conjugation which they related to the decreasing availability of thiol groups as proteins becomes cross-linked by disulphide bonds during cataractogenesis.

Inactivation of cortisol in the lens whether by protein binding or sulphate and glucormide conjugation is important since cortisol, when active, has an inhibitory effect on protein turnover. Ono, Hirano and Obara, (1972,c and 1973) related cortical inactivation to the action of the liver, adrenal gland and pituitary gland. Liver dysfunction leads to decreased protein binding and sulphate conjugation of cortisol as does hypophysectomy (in this case the trend can be reversed by addition of growth hormone) and adrenalectomy.

These results give support to earlier observations on steroid cataracts in that, if cortisol is stable in the lens, the effect would be cumulative as dose and duration of therapy increased. Also the appearance of cataract only in some treated patients may reflect variance between individuals firstly in their ability to metabolise steroids and secondly in their capacity for inactivation of cortisol within the lens. One anomaly which remains is why rats and rabbits, while having a lesser capacity to inactivate cortisol, did not produce cataract after steroid treatment in the earlier studies.

(vi) Naphthalene Cataract

A typical example of an opacity due to the influence of a toxic substance is that of naphthalene cataract. Naphthalene intake causes swelling of the lens, vacuolisation and opacification of the cortical fibres beginning in the posterior cortex. The lens increases in weight and volume due to water imbibition and becomes histologically similar to senile cataract, (Hollwick, Boateng and Kolck, 1975). Incidences of naphthalene cataract in man were recorded by Caspar, (1917) (cited by Hollwick, Boateng and Kolck, 1975) and by Ghetti and Maraini (1957) (cited by Hollwick, Boateng and Kolck, 1975) and by van Heyningen and Pirie (1967). Adams (1930) reviews the early observations made on experimentally produced naphthalene cataract and suggests that the effect is brought about by metabolic disturbance rather than by a specific toxin action. The biochemistry of naphthalene cataract formation was elucidated by van Heyningen and Pirie (1967) who implicated in the metabolism, 1, 2-Naphthaquinone, which is not included in the scheme suggested by Boyland (1963) (which they cite), but which they suggest is formed in the eye. They found that 1, 2-Naphthaquinone reacted with lens protein in vitro to form a brown stable compound and that similar compound could be extracted from the lenses of naphthalene treated rabbits. Rees and Pirie (1967) separated cortical and nuclear protein from bovine lenses and removed α-crystallin from both before adding 1,2- naphthaquinone. Nuclear proteins, which are γ-crystallin rich, precipitated quickly and all the protein from the nuclear extract had precipitated when only half that of the cortical extract had. The difference in precipitability, they suggested, is due to the thiol content of lens

proteins, that of γ-crystallin being high (Bjork, 1961) and that of α-crystallin being low (Kinoshita and Merola, 1958). Insolubilisation of lens protein in cataract is often due to SH oxidation and this was found to be the case in naphthalene cataracts where the lens opacity is reflected by an increase in insoluble protein with a concomitant decrease in sulphydryl group (Ikemoto and Iwata, 1978).

Naphthalene induced cataracts in rats and rabbits seem to be different (van Heyningen, 1979) and in the rabbit, the response to naphthalene was variable. Goldmann (1929) had induced naphthalene cataract in rat, but this work had proven unrepeatable (Koch, Doldi and Hockwin, 1976). The results of Goldmann (1929) and Lindberg (1922) (cited by Koch, Doldi and Hockwin, 1976) gave rise to the hypothesis of Koch, Doldi and Hockwin (1976) that pigmentation was significant in naphthalene intoxifications. They treated brown rats of varying pigmentation and found a correlation of pigment with degree of opacification. This correlation led them to suggest that the pigment synthesising enzyme phenol oxidase (tyrinase) is responsible for the formation of 1,2-naphthaquinone in the rat eye. In the rabbit eye, the quinone is formed by antoxidation of 1,2-dihydroxynaphthalene which may be formed in the eye from one of three precursers by three different enzymes respectively. Thus, there is a situation where the same toxic agent is reponsible for different forms of cataract in different species due to different variations in metabolism.

CHAPTER THREE

AIM OF THIS STUDY

In the two previous chapters, the structure of the lens and its protein content and the study of cataract have been described. However, investigation of the crystallins during cataractogenesis has been limited. Such investigations have been carried out for nuclear cataracts and have involved the measurement of protein insolubilisation with increasing nuclear colour (Pirie, 1968, Dilley and Pirie, 1974 and Kramps et al., 1976), and the investigation of possible mechanisms of insolubilisation such as oxidation (Truscott and Augusteyn, 1977 a, b and c and van Haard, 1980) and cross-linking (van Haard et al., 1978; and Harding, 1979). Work has possibly been concentrated on the nucleus of the lens/since opacities are easily classified according to colour while in the cortex, although the locus of the lesion is easily classified, the type of opacity i.e. whether it is due to the presence of water clefts or vacuoles or the deposition of granules, is perhaps more difficult to associate with protein changes but is linked rather to the cause; for instance diabetes is associated with anterior cortical opacities while corticosteroid induced cataract is associated with posterior cortical opacities. Some work has shown, however, that the transparency of the lens can be disrupted by the presence of high molecular weight protein aggregates (Benedek, 1971) and investigation has shown how this may be brought about (Philipson, 1969a,b and c;

1969a,b and c; Jedziniak et al., 1972, 1973 and 1975; Liem-The and Hoenders,1974; Liem-The, Stols and Hoenders, 1975; Liem-The Stols, Jap and Hoenders, 1975 and Liem-The et al., 1978).

Some attempts at the identification of the crystallins involved in cataractogenesis have been made although it has been difficult to differentiate between cataractogenic changes and those due to ageing. It has been shown immunochemically that all of the crystallins are involved in the insolubilisation process characteristic of both aging (Manski, Behrens and Martinez 1968; Manski and Martinez, 1971; Malinowski and Manski, 1980a,b and c and Manski et al., 1980) and cataractogenesis (York et al., 1972) and that, perhaps particular crystallins may be implicated in certain types of cataract e.g. β -crystallin in steroid cataract (Ono, Hirano and Obara 1972a,b and c) and γ -crystallin in naphthalene cataract (Rees and Pirie, 1967). Quantitative studies have shown disproportionate changes in the crystallins; Tapaszto (1962) and Roy and Spector (1976) found preferential loss of γ -crystallin in human cataracts while Day and Clayton (1972) found preferential loss of β - and γ -crystallin in the Cat^{Fr} hereditary cataract in the mouse, a result also found by Bours et al., (1978) studying triparanol-induced cataract in the rat.

These results have illustrated the major protein changes which may take place during cataractogenesis in particular situations and, in many cases, analyses have been carried out on pooled material using methods of crystallin separation of moderate resolving power. In this study, work has been carried out to determine whether differences,/

differences, based on the protein profiles, can be distinguished between cataracts of differing morphopathology. The hypothesis, based on the evidence that there is an ontogenic shift in the crystallin composition of successively formed fibres (Clayton, 1974) and therefore, that the protein composition varies quantitatively in differed regions of the lens (Bours, Doepfmer et al. 1976) and also with age (Bours, Weber et al 1976; Bours, 1978 and Bours et al., 1978) is that the site and nature of the lesion within the lens must be reflected by the changes in the crystallin profile. In order to prove the hypothesis, individual lenses were analysed by a high resolution technique, isoelectric focusing in dissociating conditions (as opposed to the non-dissociating conditions employed by Bours (1971) and Kramps et al. (1976)), so that quantitative measurements could be made on individual polypeptide chains in both the water soluble (W.S.) and urea soluble (U.S.) fractions of the lenses. The protein profiles of lenses of similar morphopathology could be compared for similarity of their protein content and lenses of different morphopathology compared to reveal differences between them in their protein content. Should valid subgroups be established, then, by this method, identification of differences amongst the protein profiles of lenses of similar morphopathology would be an indication to look for some aetiological factor which may have caused the differences, for instance the occurrance of some disease, such as diabetes, or the use of particular drugs, such as corticosteroids, in the patients history.

Five hundred and six lenses, which had been examined <u>in vivo</u>

to/

to obtain a detailed description of the opacities, were collected after surgery and measurements of weight made on them before separation into water soluble and urea soluble fractions. In chapter five, general information on the lenses is listed and from that, it can be seen that the information is comparable with that found in the literature although some discrepancies do arise. Having established that, for instance, the protein content of the lenses and the protein concentration of the W.S. and U.S. fractions are in agreement with previous findings comparisons of the protein profiles of lens grouped according to nuclear colour and cortical involvement are made in chapter six. Further, in chapter seven, comparisons are made between lenses of similar morphopathology but of differing aetiology; in this case involving lenses from diabetic patients, lepers and patients with retinitis pigmentosa.

In addition to this study on the protein chemistry of the lens, which is still being carried on, a more detailed survey has been going on, and is continuing, in which a detailed history, including information on the social background, medical history, ophthalmic record and clinical chemistry record, has been recorded for a population of patients and controls and stored in a computer. This survey has necessarily involved a large group of people from different disciplines - medicine, ophthalmology, clinical chemistry, statistics and biology - and the collection and handling of the data was not carried out personally although the handling of some of the computer results was. The results of this survey to date are/

are considered in chapter eight and discussed in relation to some other epidemiological studies.

Considering all of this data together, it will be shown that the term "senile cataract", as a classification, has little meaning since it will be shown that the population of lenses may be subdivided according to their morphopathology and related protein chemistry and also, that a number of single variables can be associated with the incidence of cataract.

CHAPTER FOUR

MATERIALS AND METHODS

1. Lenses.

Post-operative cataractous human lenses were frozen immediately on removal to -20°C and then stored in liquid nitrogen until analysis. Accompanying the lenses were detailed morphological descriptions of the opacities which were observed by ophthalmoscope and slit-lamp microscope examination before surgery. Also available for the first four hundred from the Princess Alexandra Eye Pavilion, Edinburgh, were relevant data from the clinical history. On the initiation of an epidemiological study, a more extensive history, including data on the social and domestic background as well as the clinical history and ophthalmological record, of the patient was obtained (Appendix A). Some of the lenses included in this study were from patients involved in the epidemiological study and the information from them plus that from subsequent patients and controls (896 patients and controls constitute the population surveyed at the present time) has been analysed by computer using the Statistical Package for the Social Sciences (S.P.S.S.) program package (Nie et al., 1975). The statistical results, using the t-test or χ^2-test according to the nature of the variable, are discussed in chapter eight.

Also included in this study are a series of ten cataractous lenses from patients with retinitis pigmentosa. These lenses were transported on CO_2 snow from various parts of the country and then stored/

stored in liquid nitrogen until analysed.

Thirty four lenses from Korea, all but one from a leper colony, plus lenses from India, Sri-Lanka, Lebanon and Libya (the latter four groups being only mentioned since analysis is not complete) were transported in saturated ammonium sulphate.

2. Preparation of the Lenses.

Lenses were weighed (excluding those transported in saturated ammonium sulphate) and homogenised individually in 1ml. 0.01M phosphate buffer, pH 7.2, containing 10mM β-mercaptoethand (βME). Those lenses transported in saturated ammonium sulphate were dialysed against the same buffer after homogenising in order to remove the ammonium sulphate. The homogenates of the lenses which were weighed were also weighed and 20ul aliquots were weighed wet, dried in an oven and then weighed dry. In this way the dry weight and subsequently the percentage dry weight of the lens could be calculated.

$$\text{Dry weight of homogenate} = \frac{\text{Wet weight of homogenate}}{\text{Wet weight of 20ul aliquot}} \times \text{Dry weight of 20ul aliq}$$

Dry weight of lens = Dry weight of homogenates - Dry weight of 1ml buffer.

This procedure was carried out in duplicate for each lens and the average taken as the value for the dry weight. The remaining homogenate was used to prepare the water soluble and urea soluble protein fractions (Figure 4.1).

Each homogenate was centrifuged for 10 minutes at 10,000g and the supernatant retained as the water soluble (W.S.) fraction. The remaining pellet was washed six or seven times in the buffer to ensure exhaustive extraction of the W.S. proteins. This was sufficient to reduce the amount of protein extracted to < 0.5mg/ml as ascertained using/

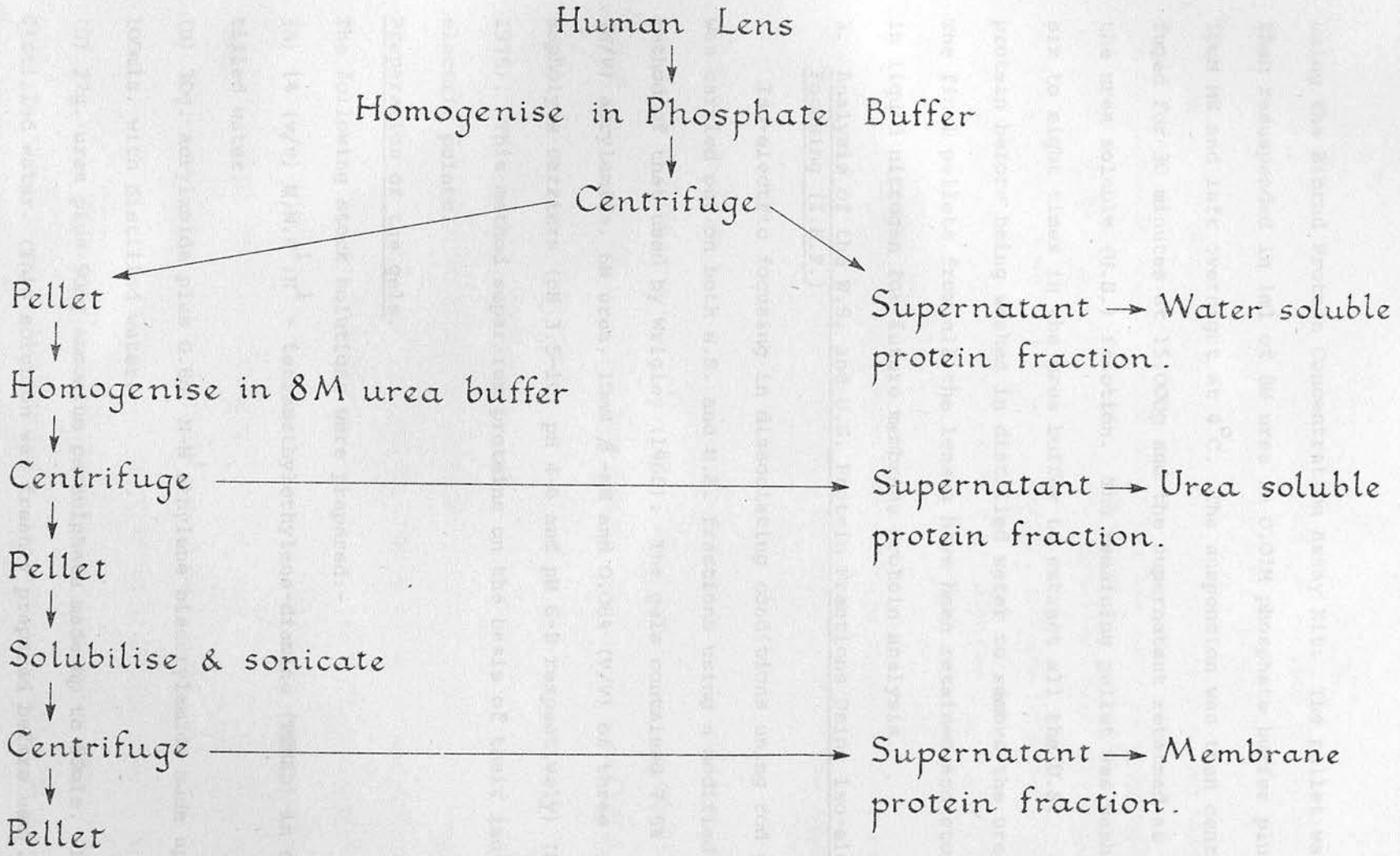


Fig. 4.1 Protocol for the preparation of the water soluble and urea soluble fractions of human cataractous lenses.

using the Biorad Protein Concentration Assay Kit. The pellet was then resuspended in 1ml of 8M urea in 0.01M phosphate buffer plus 10mM ME and left overnight at 4°C. The suspension was then centrifuged for 30 minutes at 15,000g and the supernatant retained as the urea soluble (U.S.) fraction. The remaining pellet was washed six to eight times in the urea buffer to extract all the U.S. protein before being washed in distilled water to remove the urea. The final pellets from all the lenses have been retained and stored in liquid nitrogen for future membrane protein analysis.

3. Analysis of the W.S. and U.S. Protein Fractions Using Iso-electric Focusing (I.E.F.)

Iso-electric focusing in dissociating conditions using rod gels was carried out on both W.S. and U.S. fractions using a modified method of that used by Wrigley (1968). The gels contained 7.5% (W/V) acrylamide, 6M urea, 10mM β-ME and 0.08% (V/V) of three ampholyte carriers (pH 3.5-10, pH 4-6 and pH 6-8 respectively) (Burns, 1975). This method separates proteins on the basis of their iso-electric points.

Preparation of the gels.

The following stock solutions were prepared:-

(A) 1% (v/v) N,N,N^1,N^1 - tetramethylethylene-diamine (TEMED) in distilled water.

(B) 30g. acrylamide plus 0.8g. N-N' ethylene bisacrylamide made up to 100mls. with distilled water.

(C) 27g. urea plus 50mg ammonium persulphate made up to 50mls. with distilled water. (This solution was freshly prepared before use).

The gel mixture was prepared by mixing 0.8ml. 1% TEMED, 8.0ml ammonium/

ammonium persulphate-urea solution and 0.1ml of each of the ampholines of the ranges pH 3.5-10, pH 4-6 and pH 6-8. Finally 3.0ml 30% acrylamide solution was added, and the gel mixture degassed before pouring. The gel mix was pipetted into glass tubes of 0.5mm internal diameter, which were sealed at one end with plasticine and marked so that the gels would be 9.5cm in length, and overlayed with iso-butyl alcohol so that a flat interface was achieved.

Preparation of I.E.F. Apparatus.

The upper tank (anode) electrode solution consisted of 0.2% sulphuric acid containing 10mM β ME. The reducing agent was added to prevent reassociation of the subunits. The lower tank (cathode) electrode solution consisted of 0.4% ethanolamine containing 10mM β ME. Twenty four gels in their tubes were placed vertically between the upper and lower tanks so that the ends of the gels were equidistant from the platinum electrodes.

Iso-electric Focusing Procedure.

A pre-run solution was prepared as follows - 150mg sucrose 0.1ml Ampholine (pH 3.5-10) and 31.2ul β ME made up to 4ml with distilled water. 50ul of this solution was applied to the surface of each gel and an electric current of 0.5 mA/gel applied for thirty minutes. The current ran from the anode to the cathode.

A dissociation solution was prepared so that it consisted of 11M urea, 100mM β ME and 10% sucrose (W/V) in distilled water. Equal amounts of protein sample and dissociating solution were mixed and added to the gels after the pre-run. Approximately 400µg of protein were run per gel and aliquots of the W.S. and U.S. fractions were run simultaneously./

simultaneously. An electric current of 0.5 mA/gel was applied for eighteen hours to allow complete separation and focusing. By the end of the run, the current drops to <1mA for the whole tank while the voltage increases from 45 to 450+ volts. The pre-run and the run proper were carried out at 4°C.

Staining of the Gels.

The gels were removed from the glass tubes after 18 hours of focusing and fixed in 10% trichloroacetic acid (TCA) for 30 minutes at 65°C. This procedure fixed the proteins and also removed the ampholyte carriers from the gels. The removal of the ampholytes is necessary to ensure a clear background after staining.

After thirty minutes the gels were transferred to a staining solution consisting of 0.2% Coomassie brilliant blue R (W/V) in a 45% ethanol : 45% H_2O : 10% glacial acetic acid mixture. Staining was carried out for 30 minutes at 65°C.

Destaining of the gels, to remove the background stain was carried out at 65°C in a 25% ethanol : 65% H_2O : 10% glacial acetic acid mixture, replacing the mixture as necessary. Completion of the de-staining process was carried out in 7% acetic acid (v/v) at 65°C and the gels stored at room temperature in 7% acetic acid (v/v).

Scanning of the Gels.

The stained gels were scanned on a Kipp and Zonen/Skalar KS3 microdensican and traces obtained on a Bryans XY Recorder 25000 A4. Simultaneously the areas under the peaks were integrated and expressed as a percentage of the total area under the graph. The traces for fractions (either W.S. or U.S.) from lenses of similar morphopathology were/

were superimposed and/composite standard trace obtained by drawing the curve through the mean values for each peak and trough. In order to facilitate identification of the peaks, the traces were divided arbitrarily into six areas and the bands numbered within these areas. Mean percentage values for the areas under the peaks were also calculated to allow statistical analysis.

4. Electrophoresis in Sodium Dodecyl Sulphate (S.D.S.) Polyacrylamide Gels.

This procedure, based on that of Araki and Okada (1978), separates protein on the basis of molecular weight and uses slab rather than rod gels.

Preparation of the gel.

The following stock solutions were prepared:-

(A) 30g acrylamide plus 0.8g N-N'ethylene bisacrylamide made up to 100 mls. with distilled water.

(B) 0.75M Tris (hydroxymethyl) aminomethane (TRIS) pH 8.8.

(C) 0.25M TRIS pH 6.8.

(D) 10% W/V Sodium laurylsulphate (S.D.S.)

(E) 10% W/V Ammonium persulphate.

The gel consists of two phases, one upper or stacking gel and one lower separating gel. The lower gel is made by mixing 20mls. 30% acrylamide solution, 25ml. 0.75M TRIS, 0.5ml. 10% S.D.S., 9ml. H_2O, 0.2ml. 10% ammonium per sulphate and 20ul TEMED. The upper gel is made by mixing 2.5ml. 30% acrylamide, 5ml. 0.25M TRIS, 0.25ml. 10% S.D.S., 17ml. H_2O, 0.1ml. 10% ammonium persulphate and 10ul TEMED.

Two glass plates were prepared by sealing three sides with a gasket and the lower gel mix poured to a depth of 14cm. This was overlayed/

overlayed with water until set and then the upper gel mix was used to replace the overlay and an appropriately sized comb positioned so that wells were formed for protein application.

Preparation of the S.D.S. Apparatus.

The gasket and comb were removed from between the glass plates and the gel with glass plates positioned vertically in an electrophoresis tank. The buffer was the same in each reservoir and had a final composition of 2M glycine, 0.025M TRIS and 0.1% W/V S.D.S. The upper reservoir was the cathode and the lower tank the anode.

Electrophoresis Procedure.

A sample buffer whose composition was 0.025M TRIS, 2% (W/V) S.D.S., 10% (V/V) glycerol, 5% (V/V) β ME and 0.0002% bromopherol blue was prepared. Equal volumes of sample buffer and protein sample were mixed and placed in a boiling water bath for 90 seconds and then applied to the wells. A current (running from anode to cathode) of 10mA was applied to the gel while the protein passed through the stacking gel. Once the protein had stacked, the current was increased to 30mA and separation was completed after approximately 5 hours.

Staining of the Gel.

This was carried out as for I.E.F. gels except that the gels were not fixed but placed immediately in stain.

5. Two-Dimensional I.E.F./S.D.S. Gel.

This procedure is a combination of the previous two methods whereby an I.E.F. rod gel is sliced longitudinally and a slice embedded in 1% agar above an S.D.S. slab gel. The methods for running the first demension (I.E.F.) and the second dimension (S.D.S.) are as described except that the I.E.F. gel is not fixed or stained.

6. Gel Filtration Chromatography.

Water soluble proteins were prepared from whole human lenses to give a final concentration of approximately 50mg/ml. The protein was separated into molecular weight fractions by gel filtration using either Sephadex G.100 or Utrogel AcA 54 poured in a 2.5 x 100 Pharmacia column. Elution was carried out using a 1% (W/V) ammonium bicarbonate solution at a pump rate of 1.4-2x10 on a Varioperpex pump. This allowed a fraction collection of approximately 10ml. per twenty minutes.

CHAPTER FIVE

RESULTS

CLASSIFICATION OF CATARACT GROUPS AND TESTING OF GENERAL PARAMETERS.

The description of the morphological appearance of the opacities of the lenses analysed in this study was carried out in vivo by the surgeons concerned with the aid of the ophthalmoscope and the slit-lamp microscope. The lenses could then be classified according to nuclear colour, if any, and cortical involvement. Because the nucleus of the lens, when affected by cataract, appears coloured homogeneously, a single classification is required; this may be white, yellow, brown or dark brown. The cortex, however, may be affected by a number of opacities differing in appearance (cuneiform, coronary, punctate and cupuliform) and position (anterior polar or posterior polar). These lesions may also differ in morphology involving water clefts, vesicles or granules and they are not mutually exclusive. The degree of cortical involvement may be described as immature, mature or hypermature and in some cases, these descriptions are used without any details of morphology. A breakdown of the lens groupings and the number of lenses per group is provided in table 5.1. The group termed miscellaneous includes those lenses with multiple cortical involvement or those named plus punctate opacities since, individually, these groups are made up of only one or two lenses.

The lenses were catalogued with respect to morphology along with information on the age and sex of the patients and the wet and dry weights and percentage dry weight of the lenses themselves. In this way, the groups could be tested for matching of these parameters by using the appropriate statistical tests.

TABLE 5.1 CATARACT DESCRIPTION AND INCIDENCE

CORTICAL INVOLVEMENT	NUCLEAR COLOUR						TOTAL
	None	White	Yellow	Brown	Red Brown	Dark Brown	
None	0	1	8	34	0	1	44
Immature	16	8	24	24	1	0	73
Cuneiform	7	2	17	35	2	3	66
Cupuliform	14	0	4	30	0	0	48
Cun. & Cup.	15	1	10	26	1	1	54
Mature	58	5	5	45	1	0	114
Hypermature	8	1	0	0	0	0	9
Anterior	2	1	2	6	1	0	12
Ant. & Cup.	6	0	1	7	0	0	14
Miscellaneous	16	15	10	31	4	0	76
TOTAL	142	34	81	238	10	5	510

Sex

In order to determine whether any type of cataract showed a sex bias, lenses were grouped in two ways to obtain suitable numbers for testing; in the first case according to nuclear colour regardless of cortical involvement (Table 5.2a) and secondly according to cortical type regardless of nuclear colour (Table 5.2b). Because there are minor groups not included in the latter case, the numbers are smaller than in the former which, in fact, represents the total population.

Cataract Type			
No nuclear colour	88	54	142
White nucleus	20	12	32
Yellow nucleus	55	26	81
Brown nucleus	152	85	237
Dark brown nucleus	8	6	14
	323	183	506

(a) $\chi^2 = 0.93$ Not stat. sig.

Cataract Type			
No cortical involvement	35	8	43
Immature cortical	44	27	71
Cuneiform	43	23	66
Cupuliform	28	20	48
Cuneiform + cupuliform	30	21	51
Mature cortical	67	45	112
	247	144	391

(b) $\chi^2 = 7.80$ Not stat. sig.

Table 5.2 Sex distribution of cataract patients based on (a) nuclear colour and (b) cortical involvement.

The ratio of females to males in the total population considered was 1.8 : 1 and, while some smaller groups would show a marked variation from this, overall this was consistent throughout the larger groups as shown by the χ^2 test in table 5.2(a) and (b).

Age

For the total population (minus one whose age was not known) the average age at the time of extraction was 71.4 years with a

standard deviation of 12.2 years. However, the male population is significantly younger ($p < 0.0005$) than the female population (Table 5.3) and this is illustrated by the histogram in figure 5.1. This phenomenon obtains in the smaller groupings.

TABLE 5.3 Comparison of the average ages of the male and female population at the time of cataract extraction.

Sex	No.	Mean Age (Years)	S.D.
Males	183	68.21	12.83
Females	322	73.19	11.41

Grouping the lenses as stated previously (table 5.4(a) and (b)) one group in each table - white nuclear cataract and cupuliform cataract respectively - shows a significantly earlier age of extraction; $P < 0.01$ and $P < 0.05$ respectively as calculated by means of analysis of variance. It may be that, in the latter case, the late age of extraction of hypermature cataracts also contributes to the statistical significance. However, the early age of extraction of the white nuclear and cupuliform cataracts might be expected since these two lesions would have a more immediate effect of impairment of vision than other opacities. Since no information on the age of onset or the rate of development of cataract is available, no comparison can be made with the assertion of Bellows and Bellows (1974) who stated that cupuliform cataract occurs at a younger age than other types.

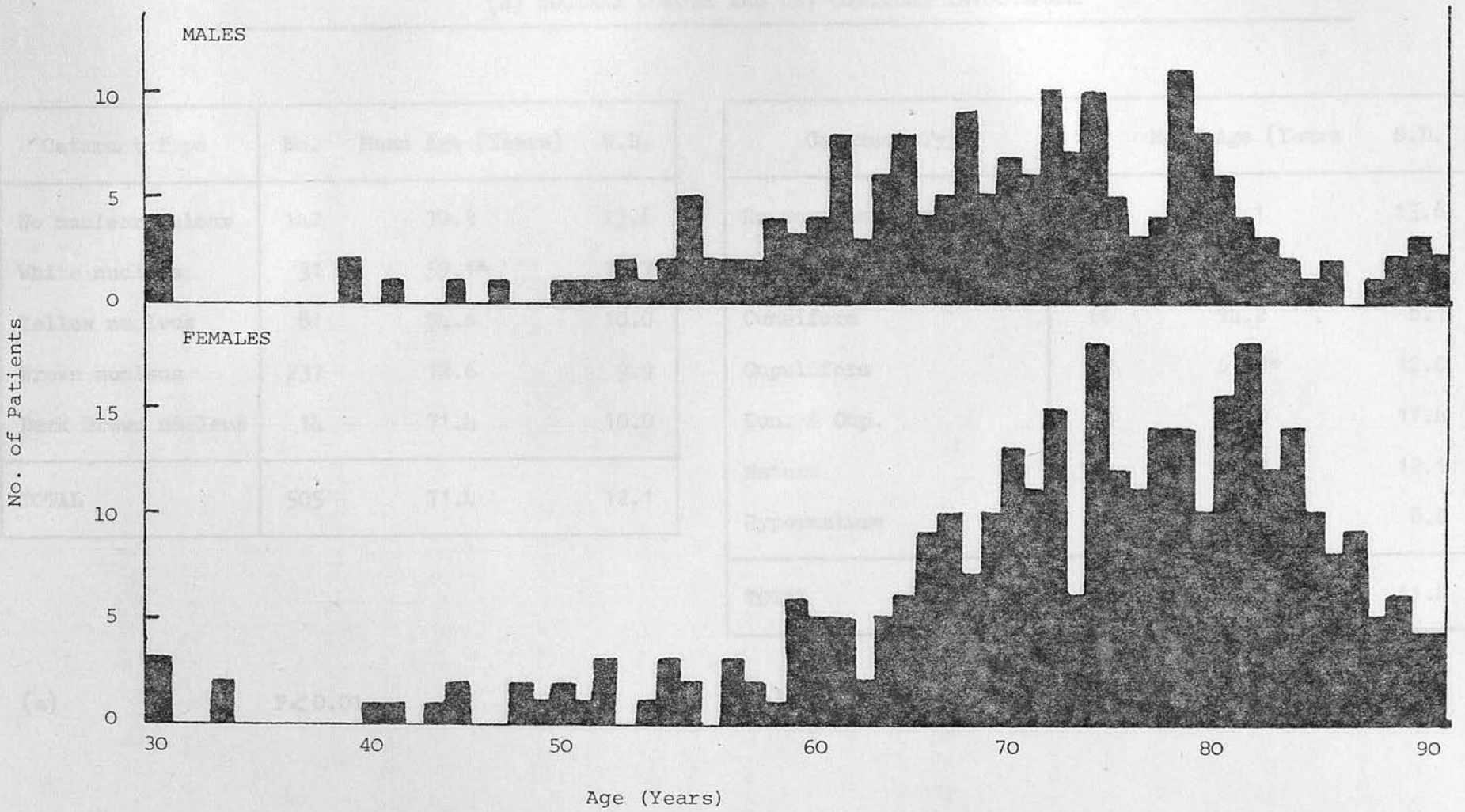


Fig. 5.1 Age distribution of male and female cataract patients. The male population is significantly younger ($p < 0.001$) with a mean age of 65.9 years while the female population has a mean age of 71.8 years.

TABLE 5.4 COMPARISON OF MEAN AGES OF PATIENTS AT TIME OF CATARACT EXTRACTION BASED ON (a) NUCLEAR COLOUR AND (b) CORTICAL INVOLVEMWNT

Cataract Type	No.	Mean Age (Years)	S.D.
No nuclear colour	142	70.3	13.6
White nucleus	31	59.1*	18.7
Yellow nucleus	81	74.6	10.0
Brown nucleus	237	72.6	9.9
Dark Brown nucleus	14	71.4	10.0
TOTAL	505	71.4	12.1

(a) $P < 0.01$

Cataract Type	No.	Mean Age (Years	S.D.
No cortical involvement	48	73.1	13.6
Immature	70	72.7	9.6
Cuneiform	66	74.2	8.7
Cupuliform	52	67.8*	12.0
Cun. & Cup.	53	74.0	11.4
Mature	114	70.7	12.1
Hypermature	8	77.1	8.0
TOTAL	411	72.1	11.4

(b) $P < 0.05$

Wet Weight, Dry Weight and Percentage Dry Weight

It has been suggested (Hockwin, et al (1971)) that the age of the lens can be determined by measuring the wet weight which increases with age (Mehta and Maisel, 1966). While this may be the case for normal lenses, in this study of human cataractous lenses no such relationship is exhibited. This lack of correlation might be expected since in some cataracts, there is an abnormal uptake of water due to osmotic imbalance, while in other cortical types, it may be the case that water loss takes place (Maraini and Mangili, 1973b). It may also be the case that these processes occur at different rates in contralateral lenses so that the two lenses of one individual have different wet weights.

Alternatively, since the lens is continuously growing, i.e. synthesising protein with a low turnover rate and laying down new layers of cells, the dry weight of the lens might be used as a measure of age since it will not be affected by water loss or uptake. However, in the case of cataractous lenses, it may be that protein synthesis is affected at the site of the lesion as suggested by Friedburg et al (1974), Koch (1976) and Ohrloff et al (1978) or that cell differentiation is affected (McAvoy and van Heyningen, 1974). In this study, no relationship is observed between dry weight of the lens and age.

Table 5.5. shows comparisons of some of the data found in this study with those of Maraini and Mangili (1973) and although a number of discrepancies can be seen in the wet and dry weight particularly - the trends are similar.

Wet Weight

When lenses are grouped according to nuclear colour, if any, but differing in cortical involvement (Table 5.6), there is a

TABLE 5.5 COMPARISON BETWEEN DATA OF MARAINI AND MANGILI (1973) AND SIMILAR DATA FROM THIS STUDY

CATARACT TYPE	NO. CASES	WET WT.	DRY WT.	% DRY WT.
Nuclear Cataract	* 9	225 ± 20	69 ± 8	30 ± 3
	29	201 ± 7	66 ± 5	31 ± 2
Immature Cortical	*15	208 ± 3	69 ± 9	33 ± 3
	16	164 ± 13	51 ± 5	31 ± 2
Cupuliform	* 6	195 ± 12	60 ± 2	29 ± 4
	13	192 ± 8	61 ± 6	33 ± 3
Mature	*10	198 ± 32	45 ± 10	22 ± 4
	35	175 ± 6	51 ± 3	28 ± 1

* DATA FROM MARAINI AND MANGILI (1973)

TABLE 5.6 WET WEIGHT

CORTICAL INVOLVEMENT	NUCLEAR COLOUR					Stat. Sig.	TOTAL
	None	White	Yellow	Brown	Dark Brown		
None	-	-	205 ± 16	201 ± 7	-	None	203 ± 7
Immature	164 ± 13	190 ± 20	206 ± 6	186 ± 9	-	None	188 ± 5
Cuneiform	214 ± 8	-	222 ± 12	193 ± 7	-	None	203 ± 5
Cupuliform	192 ± 8	-	205 ± 29	177 ± 8	-	None	184 ± 6
Cun. & Cup.	201 ± 11	-	206 ± 14	194 ± 5	-	None	198 ± 5
Mature	175 ± 6	174 ± 23	200 ± 10	174 ± 7	-	None	176 ± 4
Hypermature	156 ± 12	-	-	-	-	None	167 ± 15
Anterior	-	-	-	196 ± 15	-	None	198 ± 12
Ant. & Cup.	193 ± 22	-	-	159 ± 14	Red Brown / Dark Brown	None	177 ± 12
Miscellaneous	173 ± 9	189 ± 9	179 ± 9	184 ± 8	106 ± 7 / 208 ± 15	None	186 ± 4
Stat. Sig. (exc. misc.)	P<0.05	None	None	P<0.05	None		P<0.01
TOTAL	180 ± 4	187 ± 8	204 ± 4	185 ± 3	207 ± 7	P<0.01	

statistically significant difference in wet weight between groups of lenses with brown nuclei but different cortical involvement ($P<0.05$) and similarly between groups of lenses with no nuclear colour but different cortical involvement ($P<0.05$); those lenses with anterior or cuneiform opacities have a high wet weight and lenses with mature cortical involvement have a low wet weight. Although not statistically significant, a similar trend may also be observed in those lenses with white nuclei and those with yellow nuclei. The significance is increased, however, when lenses of similar cortical involvement are grouped together regardless of nuclear colour ($P<0.01$). (The figures for the groups termed miscellaneous are not involved in the comparisons).

No statistically significant difference in wet weight is seen between lenses of similar cortical involvement but different nuclear colour although lenses with yellow nuclei consistently have a higher wet weight. Significance is achieved if all lenses of the same nuclear colour are grouped together regardless of cortical involvement and then compared ($P<0.01$).

Dry Weight

Analysis of the dry weights (Table 5.7) of lenses grouped according to cortical involvement on a specific nuclear background shows a trend in which the dry weight is less in those lenses with mature cortical involvement. This trend is only significant ($P<0.05$) in lenses with brown nuclei and not in any other group. However, when the groups are combined so that cortical opacities are compared regardless of nuclear involvement, the difference is even more significant ($P<0.01$), there being a decrease in dry weight in mature cortical cataracts and also in lenses with anterior polar cataracts. (The figures for the groups termed miscellaneous are not involved).

TABLE 5.7 DRY WEIGHT

CORTICAL INVOLVEMENT	NUCLEAR COLOUR					Stat. Sig.	TOTAL
	None	White	Yellow	Brown	Dark Brown		
None	-	-	75 ± 6	66 ± 5	-	None	68 ± 4
Immature	51 ± 5	51 ± 8	66 ± 4	60 ± 5	-	None	60 ± 3
Cuneiform	64 ± 11	-	70 ± 4	56 ± 4	-	None	61 ± 3
Cupuliform	61 ± 6	-	68 ± 10	61 ± 5	-	None	62 ± 4
Cun. & Cup.	64 ± 5	-	63 ± 7	64 ± 4	-	None	63 ± 3
Mature	51 ± 3	39 ± 4	55 ± 5	47 ± 3	-	None	49 ± 2
Hypermature	44 ± 10	-	-	-	-	None	43 ± 9
Anterior	-	-	-	48 ± 8	-	None	50 ± 6
Ant. & Cup.	62 ± 16	-	-	52 ± 4	-	None	56 ± 7
Miscellaneous	50 ± 5	56 ± 4	51 ± 5	61 ± 4	Red 55 ± 6 Dark 59 ± 5	None	56 ± 2
Stat. Sig. (exc. misc.)	None	None	None	P < 0.05	None		P < 0.01
TOTAL	54 ± 2	54 ± 4	64 ± 2	58 ± 2	57 ± 4	P < 0.01	

If the lenses are grouped to compare dry weight of lenses with similar cortical involvement but different nuclear colour, there is no significant difference in any group although lenses with yellow nuclei consistently have a higher dry weight. However, when lenses are grouped according to nuclear colour regardless of cortical involvement there is a significant difference ($P<0.01$) in the dry weight, yellow cataracts having the highest dry weight.

% Dry Weight

The % dry weight of a lens is a measure of the relationship between the wet weight and dry weight and therefore of the water content and amount of solid material in the lens. When the % dry weight (Table 5.8) of lenses of similar nuclear involvement but differing cortical opacities are compared, no significant difference is observed although those lenses with anterior or mature cortical opacities are low in each group. When the lenses of similar cortical involvement are grouped, regardless of nuclear colour, there is significant difference in the % dry weight ($P<0.01$), these lenses with anterior and mature cortical opacities having a low percentage. It may also be observed that lenses with cupuliform involvement have a higher % dry weight although this difference is not so marked.

Lenses of similar cortical involvement but differing nuclear colour show no significant difference in % dry weight and when lenses of similar nuclear colour are grouped, regardless of cortical involvement, and these groups compared, still no significant difference is shown.

TABLE 5.8 % DRY WEIGHT

CORTICAL INVOLVEMENT	NUCLEAR COLOUR						TOTAL
	None	White	Yellow	Brown	Dark Brown	Stat. Sig.	
None	-	-	37.5 ± 2.1	31.0 ± 2.3	-	None	32.3 ± 1.8
Immature	30.6 ± 1.5	27.0 ± 4.2	33.5 ± 1.8	33.3 ± 2.0	-	None	32.3 ± 1.0
Cuneiform	29.2 ± 4.3	-	32.5 ± 2.2	29.1 ± 2.4	-	None	30.0 ± 1.4
Cupuliform	32.9 ± 2.8	-	34.2 ± 2.1	34.8 ± 1.9	-	None	34.2 ± 1.4
Cun. & Cup.	32.8 ± 3.0	-	31.8 ± 4.0	33.2 ± 2.0	-	None	32.4 ± 1.5
Mature	28.2 ± 1.5	23.6 ± 3.3	27.6 ± 2.8	28.2 ± 1.6	-	None	27.8 ± 1.0
Hypermature	27.1 ± 5.4	-	-	-	-	None	25.5 ± 5.0
Anterior	-	-	-	23.0 ± 2.1	-	None	23.0 ± 1.7
Ant. & Cup.	31.6 ± 6.3	-	-	35.3 ± 4.2	-	None	32.8 ± 3.3
Miscellaneous	31.4 ± 2.0	30.1 ± 1.9	28.7 ± 2.1	33.8 ± 1.7	Red 27.3 ± 3.0 Dark 28.6 ± 1.9	None	31.0 ± 0.9
Stat. Sig. (exc. misc.)	None	None	None	None	None		P< 0.01
TOTAL	29.7 ± 0.9	28.6 ± 1.5	32.4 ± 1.0	31.3 ± 0.8	27.8 ± 2.0	None	

Discussion

From the tables of results (Tables 5.6, 5.7 and 5.8) it can be seen that statistically significant differences may occur for each of the parameters - wet weight, dry weight and percentage dry weight - in each grouping of the lenses independently, i.e. the wet weights may show significant differences between groups of lenses without significant differences necessarily being present between the dry weights or percentage dry weights. It can also be seen from the tables that differences are present between groups differing in cortical involvement rather than nuclear involvement except, in the latter case, when they are combined. If the values of these parameters are grouped according to decreasing dry weight with respect to cortical involvement on particular nuclear backgrounds (Figures 5.2, 5.3 and 5.4) or regardless of nuclear background (Figure 5.5) or with respect to nuclear colour regardless of cortical involvement (Figure 5.6), some patterns showing the relationships of these parameters for particular groups of cataract do emerge. The possible combinations include wet weight and dry weight being both high or both low, giving similar percentage dry weights, or one being high and one being low, giving differing percentage dry weights. Thirdly, there may be one of these combinations but with a percentage dry weight which is out of line. In this way, some indication of the role of the protein : water balance in certain types of cataract may be given.

These cataracts with high wet weight include those cataracts usually associated with osmotic imbalance and water uptake, for example those affecting the anterior cortex and with cuneiform morphology (Figures 5.2-5.5)(Kinoshita, 1974) and those lenses with little cortical cataractous change eg. yellow nuclear cataracts

Fig. 5.2. Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) respectively of cataractous lenses grouped according to cortical involvement on a clear nuclear background. In this case, there is statistically significant difference (as tested by analysis of variance) between the wet weights only. The relationship of the wet, dry and percentage dry weights within groups is discussed in the text.

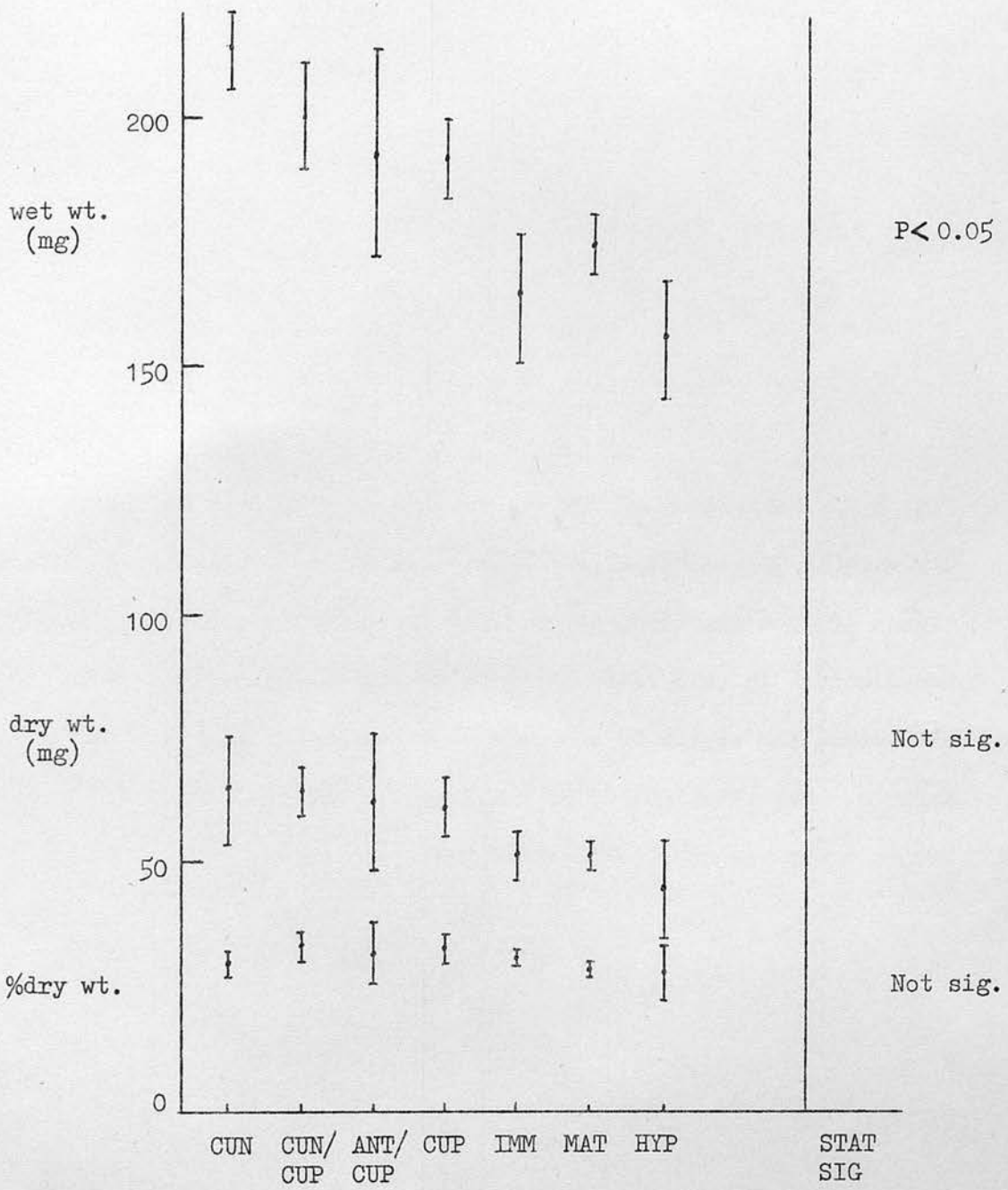

200
wet wt.
(mg)
150
100
dry wt.
(mg)
50
%dry wt.
0
P< 0.05
Not sig.
Not sig.
CUN
CUN/
CUP
ANT/
CUP
CUP
IMM
MAT
HYP
STAT
SIG

Fig. 5.2

Fig. 5.3. Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) respectively of cataractous lenses grouped according to cortical involvement on a yellow nuclear background. In this case, there is no statistically significant difference (as tested by analysis of variance) between any of the factors. The relationship of the wet, dry and percentage dry weights within groups is discussed in the text.

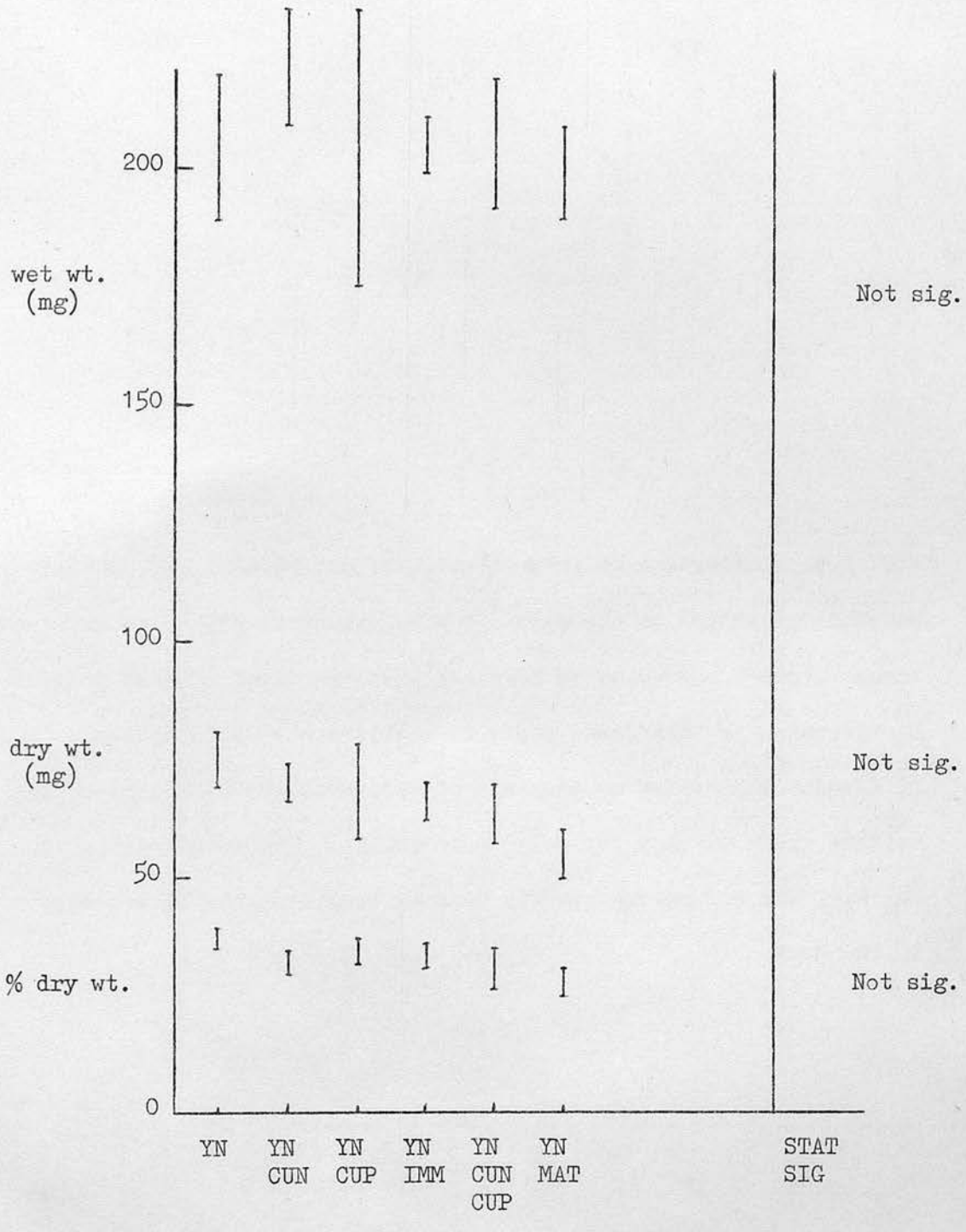

200
150
100
50
0
wet wt.
(mg)
dry wt.
(mg)
% dry wt.
Not sig.
Not sig.
Not sig.
YN
YN CUN
YN CUP
YN IMM
YN CUN CUP
YN MAT
STAT SIG

Fig. 5.3

Fig. 5.4. Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) respectively of cataractous lenses grouped according to cortical involvement on a brown nuclear background. In this case, there is statistically significant difference (as tested by analysis of variance) between both the wet weights ($p < 0.05$) and dry weights ($p < 0.05$). The relationship of the wet, dry and percentage dry weights within groups is discussed in the text.

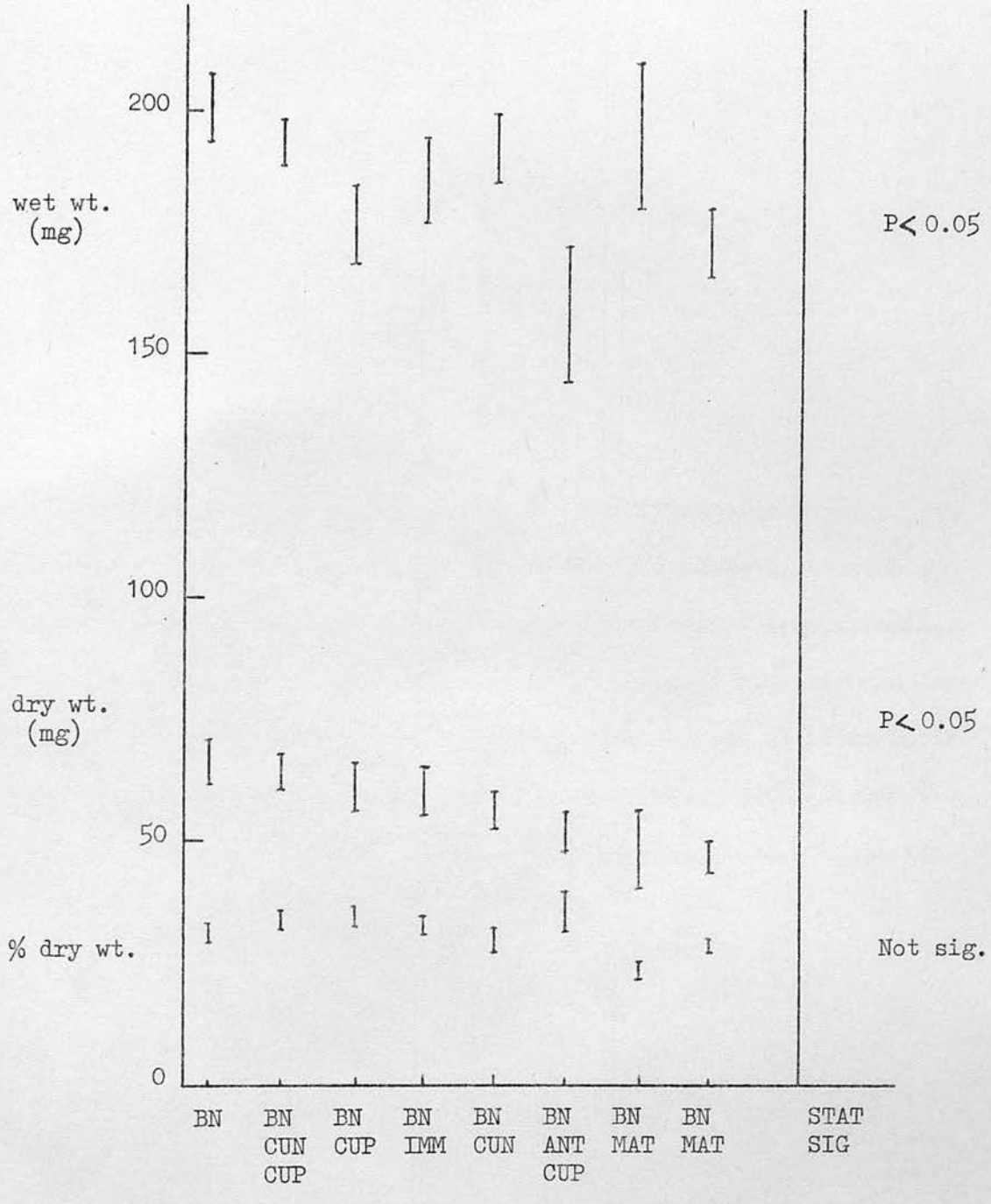

200
150
100
50
0
wet wt.
(mg)
dry wt.
(mg)
% dry wt.
P< 0.05
P< 0.05
Not sig.
BN
BN CUN CUP
BN CUP
BN IMM
BN CUN
BN ANT CUP
BN MAT
BN MAT
STAT SIG

Fig. 5.4

Fig. 5.5. Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) respectively of cataractous lenses grouped according to cortical involvement regardless of nuclear colour. In this case, all three factors exhibit statistically significant difference; $p < 0.01$ in each case (as tested by analysis of variance). The relationship of the wet, dry and percentage dry weights within groups is discussed in the text.

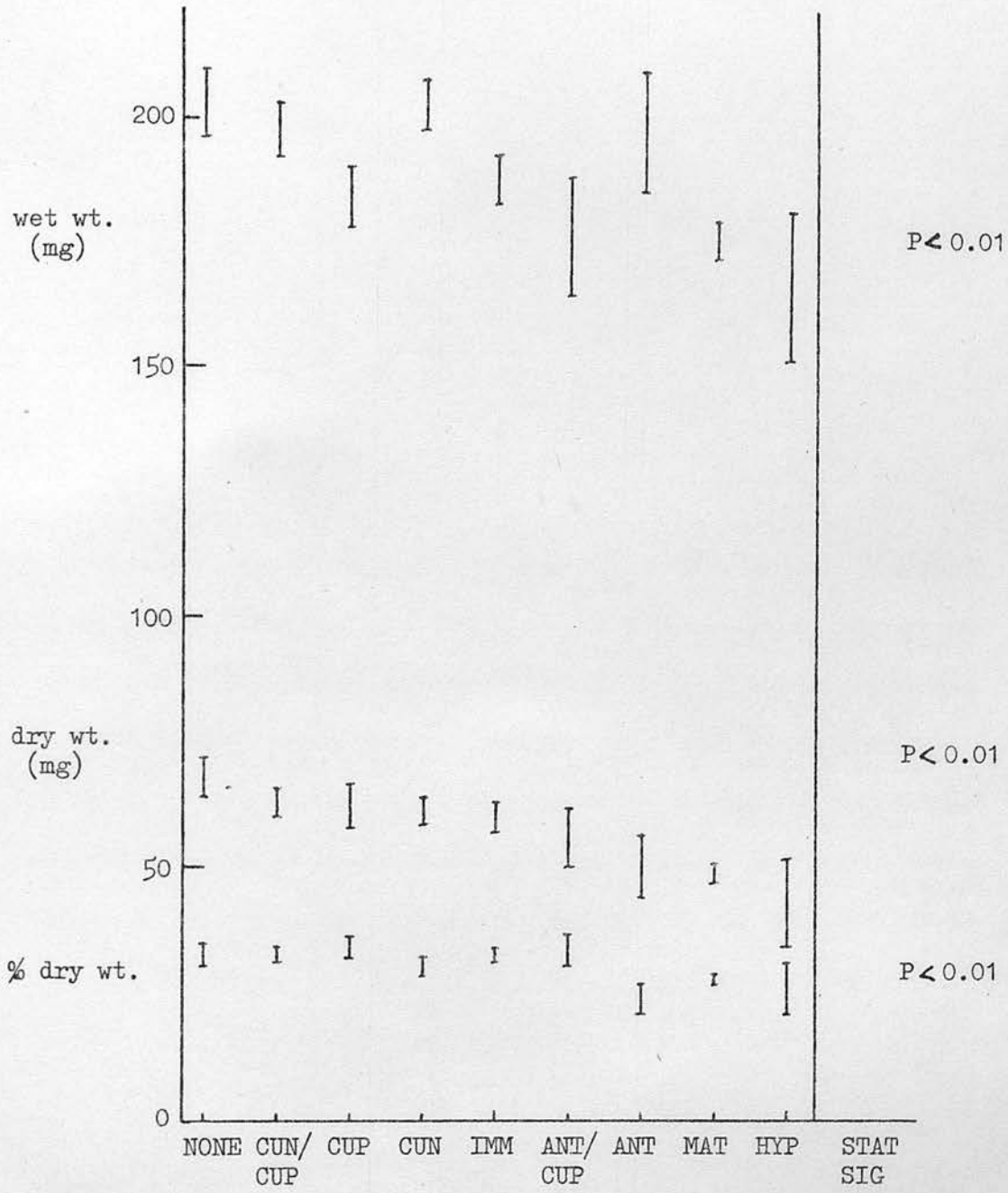


Fig. 5.5 CORTICAL INVOLVEMENT

Fig. 5.6. Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) respectively of cataractous lenses grouped according to nuclear colour regardless of cortical involvement. In this case, there is statistically significant difference (as tested by analysis of variance) between both the wet weights ($p < 0.01$) and the dry weights ($p < 0.01$). The relationship of the wet, dry and percentage dry weights within groups is discussed in the text.

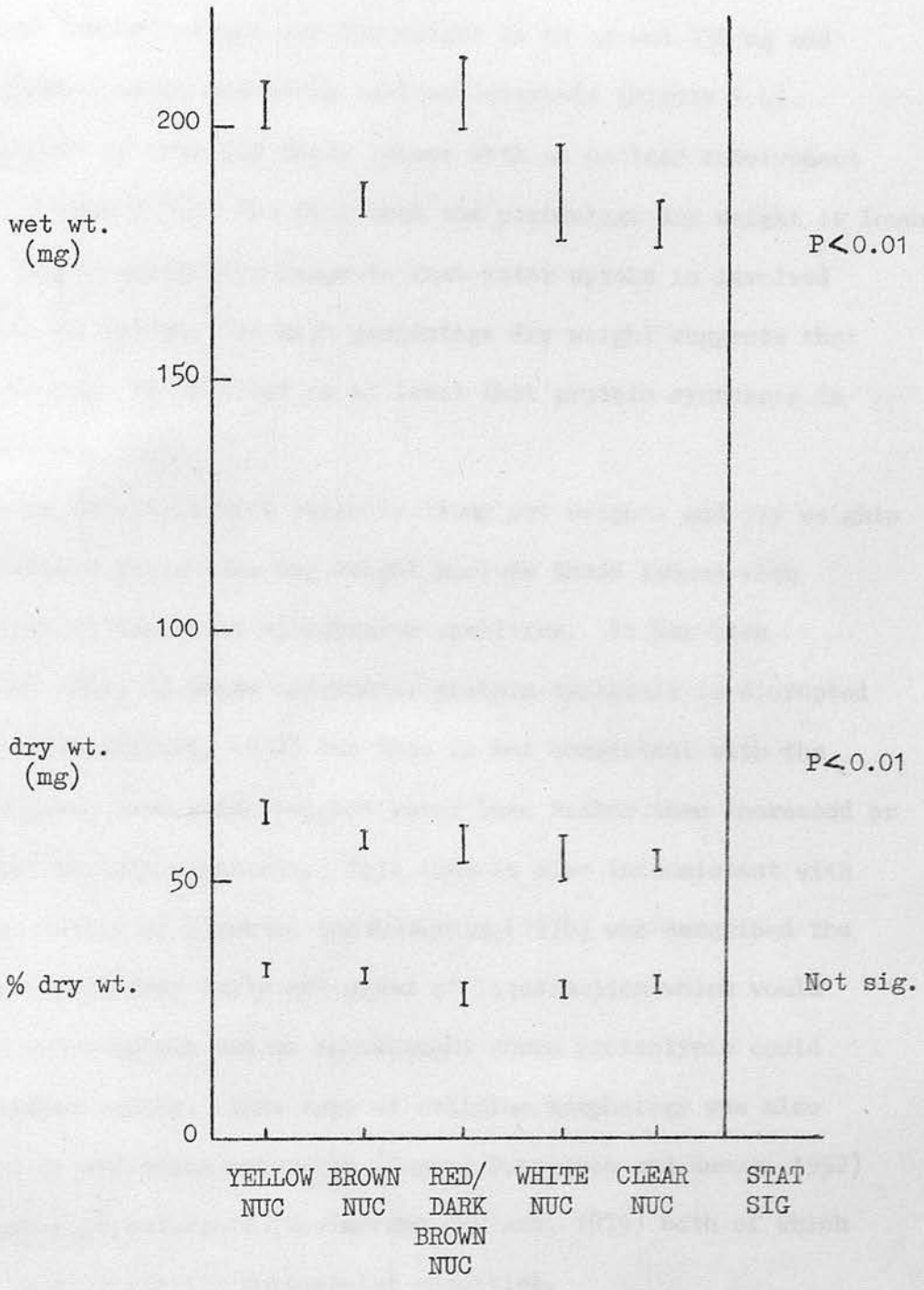

200
150
100
50
0
wet wt.
(mg)
dry wt.
(mg)
% dry wt.
P<0.01
P<0.01
Not sig.
YELLOW NUC
BROWN NUC
RED/ DARK BROWN NUC
WHITE NUC
CLEAR NUC
STAT SIG

Fig. 5.6

(Figure 5.3), whose wet weight and dry weight measurements (205 mg and 75 mg) are similar to those of normal lenses as measured by Mehta and Maisel (1966) and Maraini and Mangili (1973) both of whom find the wet weight and dry weight to be around 230 mg and 70 mg respectively, and brown nuclear cataracts (Figure 5.4). The converse is true for those lenses with no nuclear involvement at all (Figure 5.6). The fact that the percentage dry weight is lower in the former categories suggests that water uptake is involved while in the latter, the high percentage dry weight suggests that water loss may be involved or at least that protein synthesis is not affected.

Those cataracts with slightly lower wet weights and dry weights but increased percentage dry weight include those lenses with cupuliform or posterior subcapsular opacities. It has been suggested that, in these cataracts, protein synthesis is disrupted (Koch, 1976; Ohrloff, 1978) but this is not consistent with the results given here which suggest water loss rather than increased or decreased protein synthesis. This idea is also inconsistent with the observation of Streeten and Eslaghian (1978) who described the presence of bladder cells and areas of liquefaction which would suggest water uptake and an environment where proteolysis could occur rather easily. This type of cellular morphology was also observed in radiation cataracts (Cogan, Donaldson and Reese, 1952) and in steroid cataracts (Greiner and Chylack, 1979) both of which agents cause posterior subcapsular opacities.

Finally, while the trends of wet weight and dry weight tend to be parallel with regard to their decrease as cortical cataracts mature (Figures 5.2-5.5) the percentage dry weight also decreases which suggests that the total loss of material from the lens is not

an equal balance of water and dry material but a greater decrease in the latter. This suggests that in the early stages of cortical cataract some interruption of the protein metabolism occurs from which the lens never recovers. Thus, in a population matched for age, those lenses with more mature cortical opacities, assuming that these have been progressing for a longer period of time than immature or early cortical opacities, will have weight measurements more akin to those of younger lenses and indeed may have a lighter dry weight since proteolysis may have occurred as well as protein leakage both of which phenomena have been reported in the literature.

CHAPTER SIX

PROTEINS OF THE LENS.

The lens homogenates were divided, as described in the materials and methods section, into the water soluble (WS), urea soluble (US) and urea insoluble (UI) fractions; the former two only have been analysed. The protein concentration was measured in each case and the results tabulated and statistically tested using analysis of variance (Tables 6.1 and 6.2). From these results it can be seen that the protein concentration of the W.S. fractions decreases significantly in mature cortical cataracts both on specific nuclear backgrounds and regardless of nuclear background. There is, however, no difference in the W.S. concentrations when nuclear colour is considered either with specific cortical involvements or regardless of cortical involvement except when pure yellow nuclear cataracts are compared with pure brown nuclear cataracts in which case the concentration is significantly less (according to the student t-test) in brown nuclear cataracts. This is seen as a trend with some cortical morphologies e.g. in the case of cuneiform and miscellaneous cortical involvement, the protein concentration does decrease with increasing nuclear colour. Two anomalies are the low figure for yellow nuclear with cuneiform plus cupuliform cataracts in comparison with those for cataract of the same cortical morphology but different nuclear colour and the rather high figure for brown nuclear mature cortical in comparison with the other types of mature cataracts.

There is little variation, however, in the protein concentration

of/

TABLE 6.1 WATER SOLUBLE FRACTION CONCENTRATION

CORTICAL INVOLVEMENT	NUCLEAR COLOUR					STAT. SIG.	TOTAL
	NONE	WHITE	YELLOW	BROWN	RED BROWN		
None	-	-	52.12±8.72	30.00±2.29	-	P 0.05	35.20±3.44
Immature	49.93±3.68	33.67±8.41	42.76±2.56	44.70±3.69	-	N.S.	43.30±2.04
Cuneiform	43.88±8.10	-	42.61±3.81	34.68±2.99	-	N.S.	37.95±2.34
Cupuliform	38.86±3.22	-	40.25±1.75	37.95±3.14	-	N.S.	38.50±2.00
Cun.+ Cup.	41.31±4.53	-	33.00±1.89	42.25±3.65	-	N.S.	40.15±2.36
Mature	23.56±2.19	22.00±6.65	21.50±2.00	29.10±3.16	-	N.S.	25.11±1.68
Hypermature	17.83±2.45	-	-	-	-	N.S.	17.83±2.45
Miscellaneous	39.59±5.47	32.82±4.26	33.18±4.97	31.34±2.31	24.75±3.03	N.S.	33.62±1.89
Stat. Sig. (excl. MISC.)	P 0.01	N.S.	P 0.05	P 0.01			P 0.01
TOTAL	34.18±2.01	31.23±3.40	39.32±1.86	35.05±1.23	24.75±3.03	N.S.	

TABLE 6.2 UREA SOLUBLE FRACTION CONCENTRATION

CORTICAL INVOLVEMENT	NUCLEAR COLOUR					STAT. SIG.	TOTAL
	NONE	WHITE	YELLOW	BROWN	RED BROWN		
None	-	-	24.90±3.47	21.23±1.96	-	N.S.	22.25±1.71
Immature	26.36±3.08	22.67±3.29	25.92±1.76	20.71±1.52	-	N.S.	23.78±1.07
Cuneiform	17.50±2.85	-	29.62±1.70	19.60±2.28	-	P 0.05	22.23±1.67
Cupuliform	24.86±3.54	-	23.00±12.00	21.82±1.08	-	N.S.	23.00±1.61
Cun.+ Cup.	22.56±2.10	-	24.88±2.34	17.62±1.58	-	P 0.05	20.67±1.22
Mature	19.60±1.58	24.50±1.19	25.25±0.25	26.17±1.90	-	P 0.05	22.52±1.15
Hypermature	20.86±3.61	-	-	-	-	N.S.	20.83±3.61
Miscellaneous	18.66±3.10	23.77±1.88	35.88±0.40	34.44±0.81	20.35±21.45	P 0.01	27.66±1.19
Stat. Sig. (Excl. Misc.)	N.S.	N.S.	N.S.	P 0.05			N.S.
TOTAL	20.88±1.05	23.62±1.35	27.70±1.02	23.83±0.82	20.35±1.45	P 0.01	

of the U.S. fractions (Table 6.2), the variation being less with regard to cortical involvement than with regard to nuclear colour. The apparent trend is that the U.S. protein concentrations increases from no nuclear colour to yellow nucleus and then drops again as the nucleus darkens. If this is matched to the trend of decreasing W.S. concentrations it would seem that initially the W.S. protein becomes urea soluble and then urea insoluble so that the normal level of urea soluble protein for lenses of that age increases and decreases around some sort of equilibrium level. Although the urea insoluble (U.I.) fractions have been neither quantified nor analysed, it is obvious from the size and colour of the remaining pellets that this fraction increases with increasing nuclear colour, supporting the idea that as the nucleus darkens, the protein becomes urea insoluble.

These observations along with those on the wet and dry weights of the lenses suggest that the protein of the lens have, in the event of cataract, a choice of fates depending on their location. Those in the nucleus become insolubilised by some mechanism, while in the cortex, protein synthesis is disrupted and perhaps the protein itself becomes insoluble, is hydrolysed or lost from the lens by leakage. These phenomena have been well reported in the literature. Harding (1972, 1979) has demonstrated the occurrance of conformational changes followed by aggregation in human lens protein in nuclear cataracts: denaturation, crosslinking and insolubilisation of nuclear proteins has been reported by Dilley and Pirie (1974); and Kramps et al. (1976), and Tuscott and Augusteyn (1977a,b) have shown how oxidative/

oxidative changes in nuclear cataract can lead to aggregation and insolubilisation of protein. Ringens et al. (1978) have suggested that aggregation of α-, β- and γ-crystallins occurs in the cortex leading to the formation of what they term high molecular (HM) crystallin which they consider to be an intermediate stage in the insolubilisation process (Liem-The et al. 1974b, 1975a and 1978) although other workers (Spector et al. 1974 and Roy and Spector, 1976) have found this to occur more in the nucleus than in the cortex. Day and Clayton (1972), however, showed that, in the specific case of the anterior polar subcapsular cataract found in mice with the gene Cat^{Fr}, no insolubilisation of protein is associated with cataract but rather that disruption of protein synthesis was a more likely explanation for low protein levels in each of the W.S., U.S. and U.I. fractions. Piatiogorsky et al. (1980) also showed, in the Philly mouse / mutant that protein synthesis is disrupted during the development of cortical cataract. The work of Philipson (1969a, b and c) indicates clearly the drop in protein concentration in the cortex of sugar induced and X-ray induced cataracts compared with normal lenses and suggests that perhaps the disruption of fibre membranes allows the leakage of small molecular weight proteins and that, rather than the presence of macromolecular aggregates, the discontinuity of protein concentration gradients is the cause of loss of transparency. This lack of homogeneity was hinted at by Dische (1968) in a review where he also stated evidence that protein synthesis disruption occurred in both sugar induced and X-ray induced cataract as well as in tryptophane deficiency cataract; those three types of cataract/

cataract may be assumed to have different mechanisms of insult but give similar end results.

Three general observations have been made; (1) the protein balance throughout the lens differs in terms of α -, β - and γ-crystallin, (2) some or all of these proteins may be changed either quantitatively or qualitatively in the event of cataract and (3) the nature of the changes is dependent on the site of lesion within as well as on the mechanism of insult to the lens. Therefore analysis of the protein balance should give some indication as to the effect of that change. The following sections describe the analyses of cataractous lenses in terms of the water soluble and urea soluble protein profiles.

PROTEIN PROFILES OF CATARACTOUS LENSES.

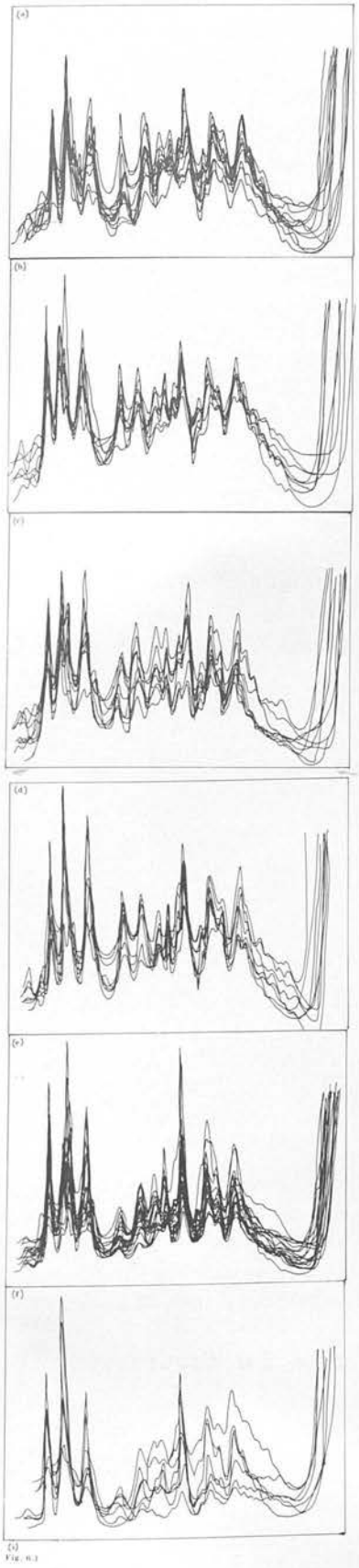
Aliquots of the W.S. and U.S. protein fractions of individual lenses were analysed by iso-electric focusing on polyacylamide rod gels as described in the materials and methods section and the gels stained and scanned so that a graphic trace was obtained for each sample along with integration values for the areas under each peak of the graph. In this section we will consider lenses of similar morphopathology, i.e. nuclear and cortical involvement, while lenses of similar medical history, e.g. lenses from patients with diabetes or retinitis pigmentosa, will be discussed in the following section.

For lenses of the same morphopathological classification the traces were superimposed so that an "average" trace could be obtained (Figures 6.1 - 6.6) and considered alongside average values for each peak which had been calculated from the individual values. While this may be thought to have the same effect as pooling the lenses in the first place, this method has the advantage of indicating the range of values for each protein band, thus allowing a more accurate statistical comparison between different types of cataract. Secondly, it allows identification of individual lenses which differ in their protein profile from lenses of similar morphopathology which may turn out to be correlated with something specific in the medical history of that patient which might have been a determinant of that cataract: for instance the incidence of certain diseases such as diabetes or the use of certain drugs such as corticosteroids.

For most cases, the numbers and positions of the protein bands were the same, allowing for the variation in the pH gradient between separate gel runs. Because of this variation, it was sometimes the case/

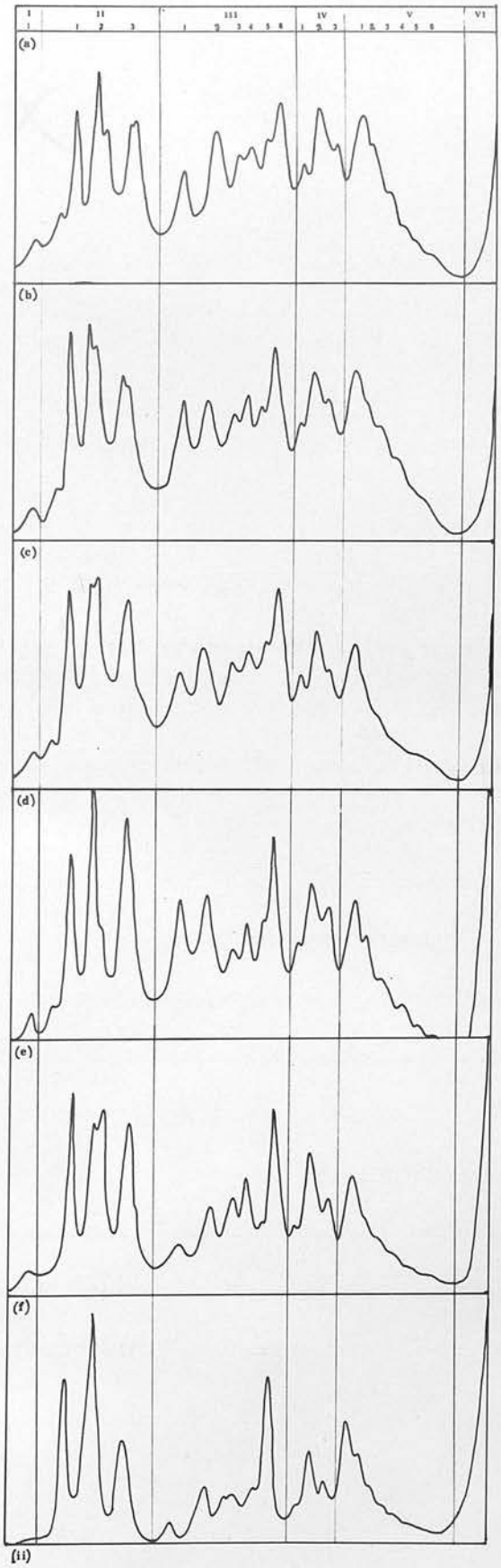
Fig. 6.1. (i) The superimposition * of densitometric traces of I.E.F. gels and (ii) the average trace derived from them for the W.S. fractions of the following cortical cataract types : (a) immature, (b) cuneiform, (c) cupiliform, (d) cuneiform plus cupuliform, (e) mature and (f) hypermature.

* Although some variation is expected within groups, the variation observed in the superimposition exercise may be exaggerated by the fact that between gel runs, variations did occur in the pH gradient of the I.E.F. gels and therefore in some gels peaks would be higher and narrower while in other gels, the same peaks would be lower and broader. The figure does illustrate, however, by means of the average traces, the differences between the protein profiles of different categories of cataract. This phenomons is true for figures 6.1 to 6.6.



(i)

Fig. 6.1



(ii)

Fig. 6.2. (i) The superimposition * of densitometric traces of I.E.F. gels and (ii) the average trace derived from them for the U.S. fractions of the following cortical cataract types : (a) immature, (b) cuneiform, (c) cupuliform, (d) cuneiform plus cupuliform, (e) mature and (f) hypermature.

* "See footnote to figure 6.1".

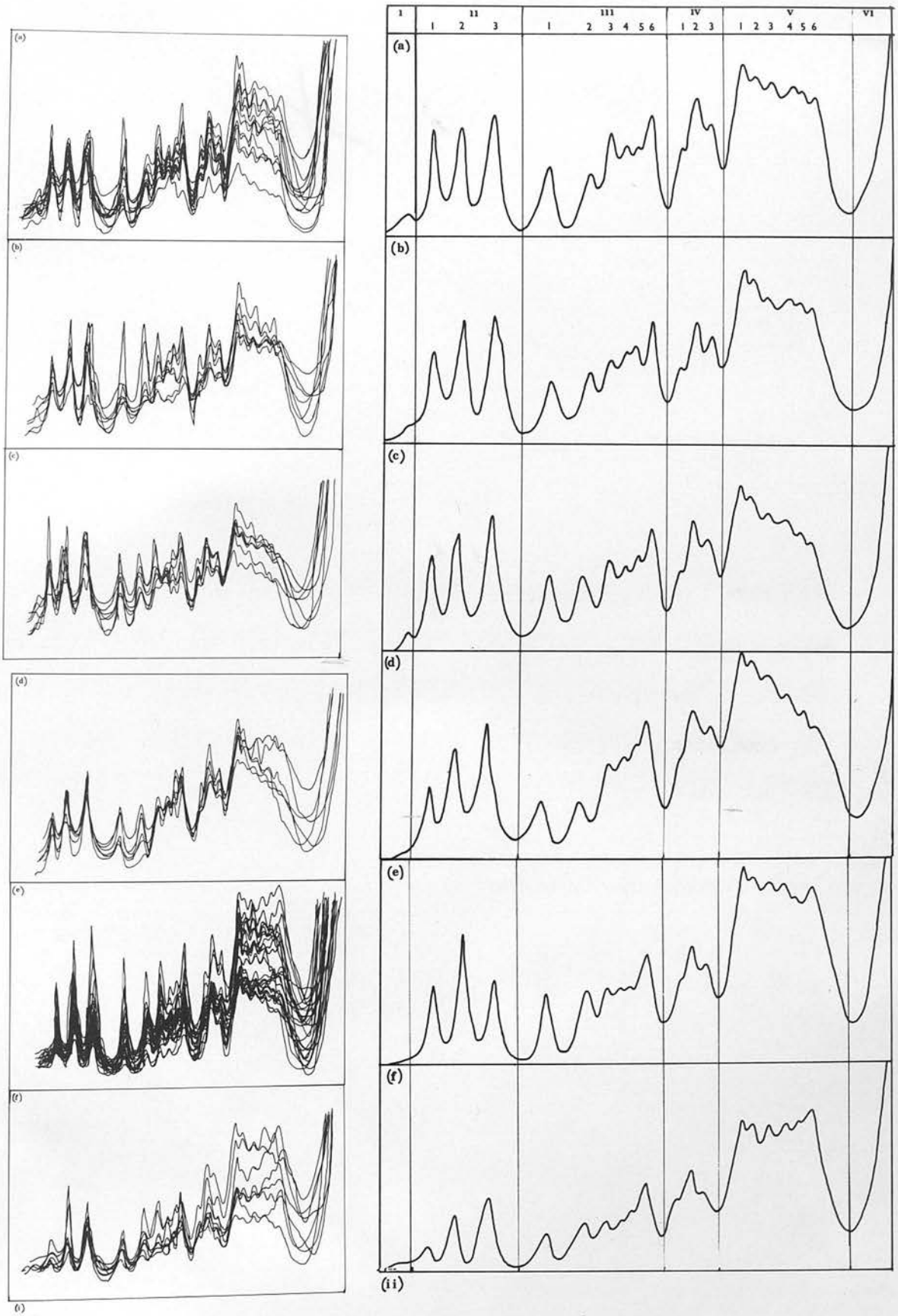


Fig. 6.2

Fig. 6.3. (i) The superimposition * of densitometric traces of I.E.F. gels and (ii) the average trace derived from them for the W.S. fractions of yellow nuclear cataracts with the following cortical involvements : (a) none, (b) immature, (c) cuneiform, (d) cupuliform, (e) cuneiform plus cupuliform and (f) mature.

* "See footnote to figure 6.1".

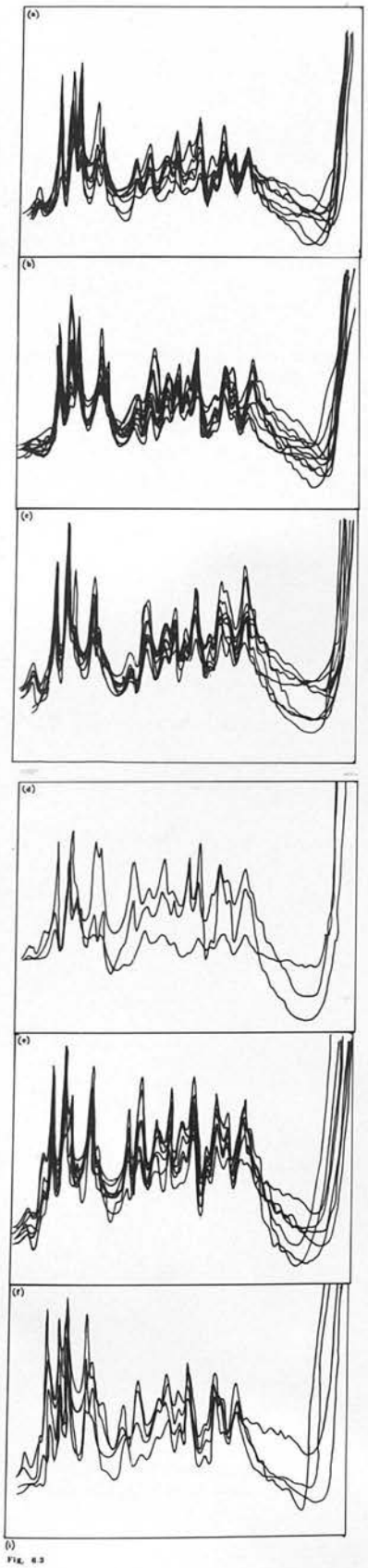


Fig. 6.3

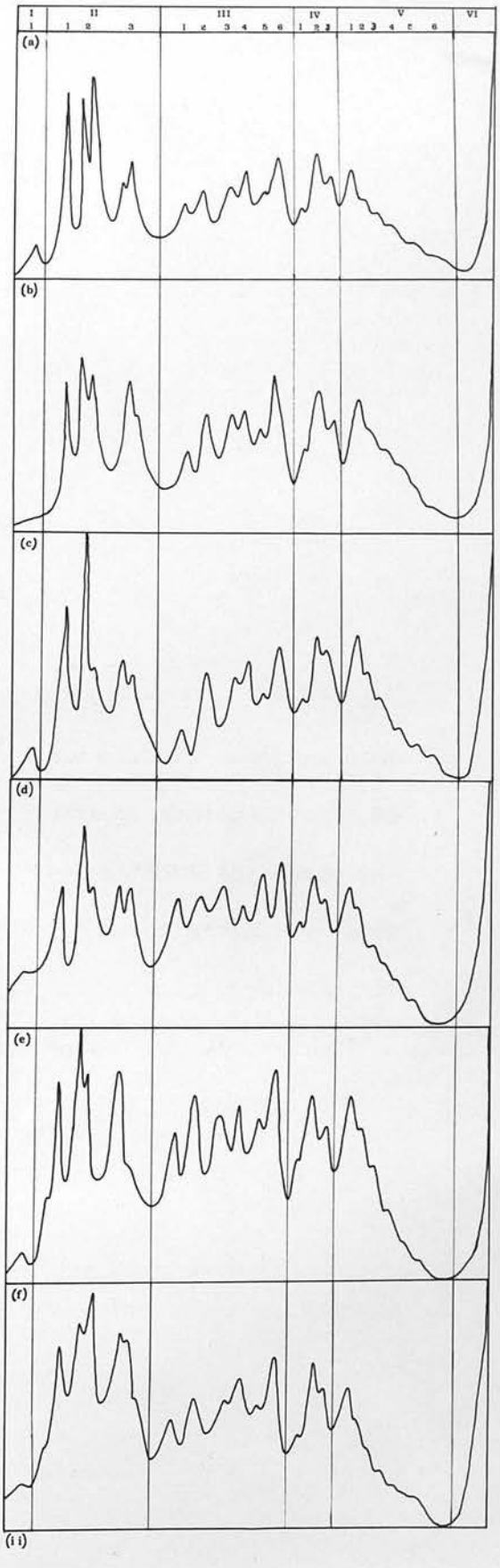


Fig. 6.4. (i) The superimposition * of densitometric traces of I.E.F. gels and (ii) the average trace derived from them for the U.S. fractions of yellow nuclear cataracts with the following cortical involvements : (a) none, (b) immature, (c) cuneiform (d) cupuliform, (e) cuneiform plus cupuliform and (f) mature.

* "See footnote to figure 6.1".

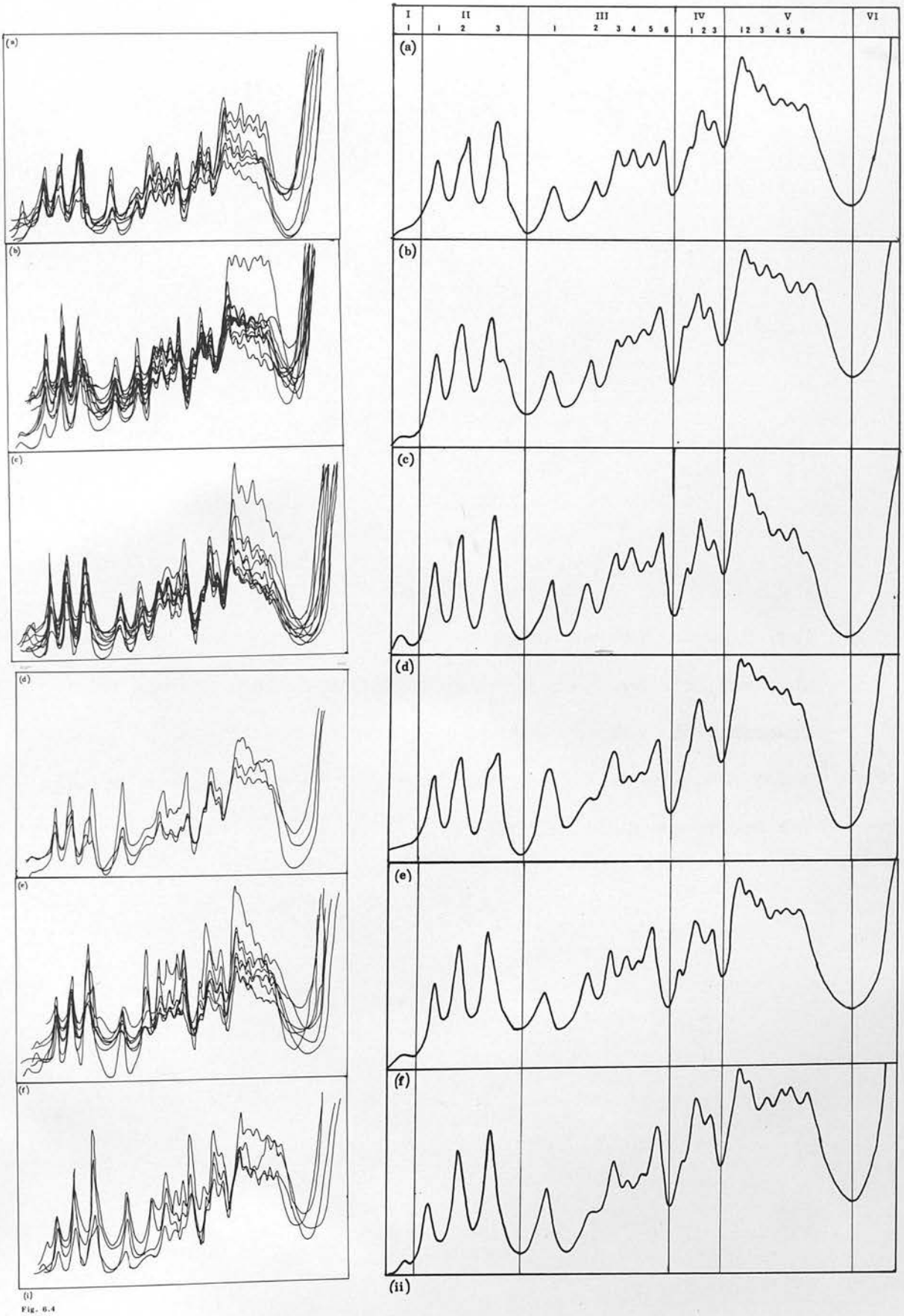
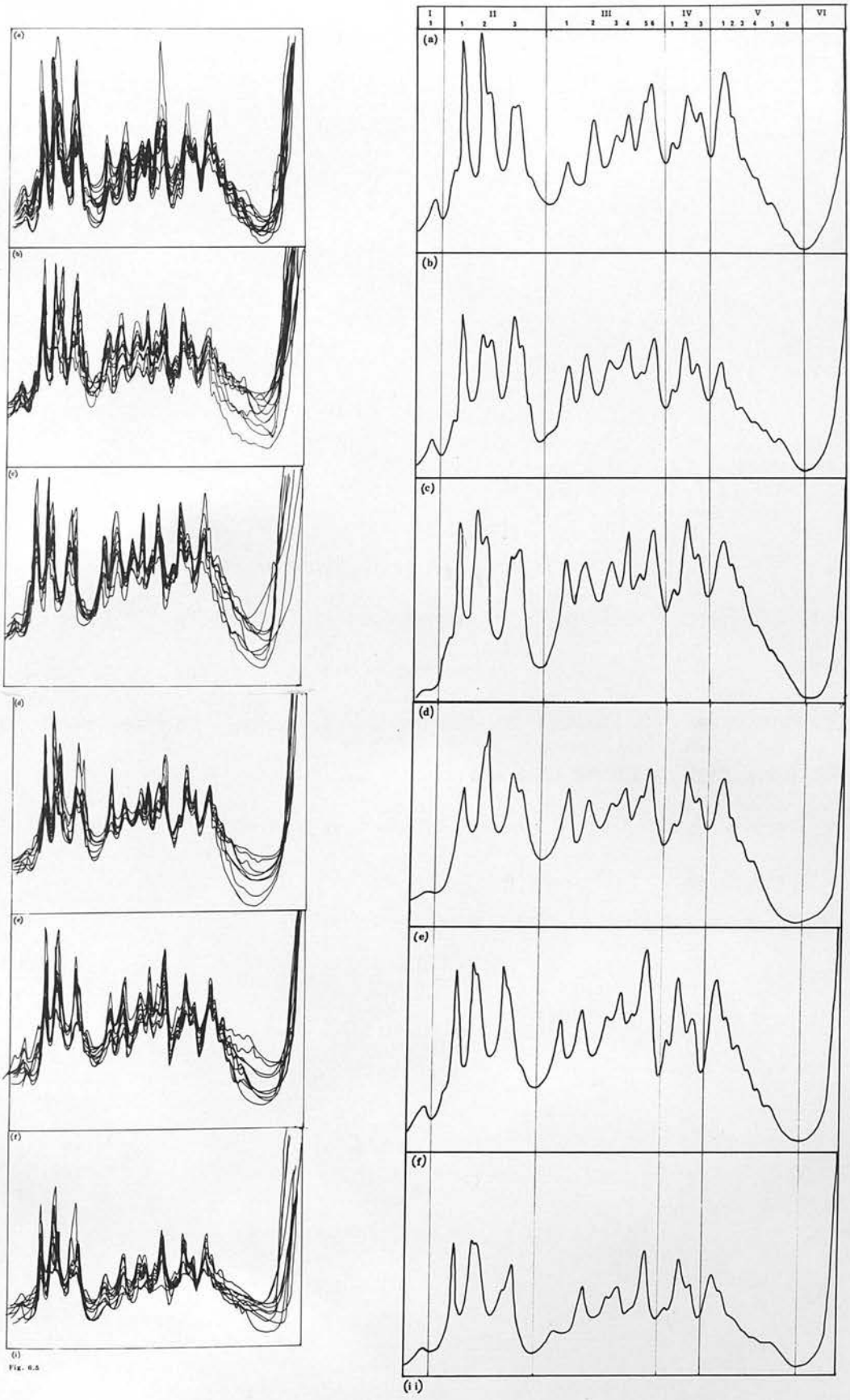


Fig. 6.4

Fig. 6.5. (i) The superimposition * of densitometric traces of I.E.F. gels and (ii) the average trace derived from them for the W.S. fractions of brown nuclear cataracts with the following cortical involvements : (a) none, (b) immature, (c) cuneiform, (d) cupuliform, (e) cuneiform plus cupuliform and (f) mature.

* "See footnote to figure 6.1".



(i) (ii)

Fig. 6.5

Fig. 6.6. (i) The superimposition * of densitormetric traces of I.E.F. gels and (ii) the average trace derived from them for the U.S. fractions of brown nuclear cataracts with the following cortical involvements (a) none, (b) immature, (c) cuneiform, (d) cupuliform, (e) cuneiform plus cupuliform and (f) mature.

* "See footnote to figure 6.1".

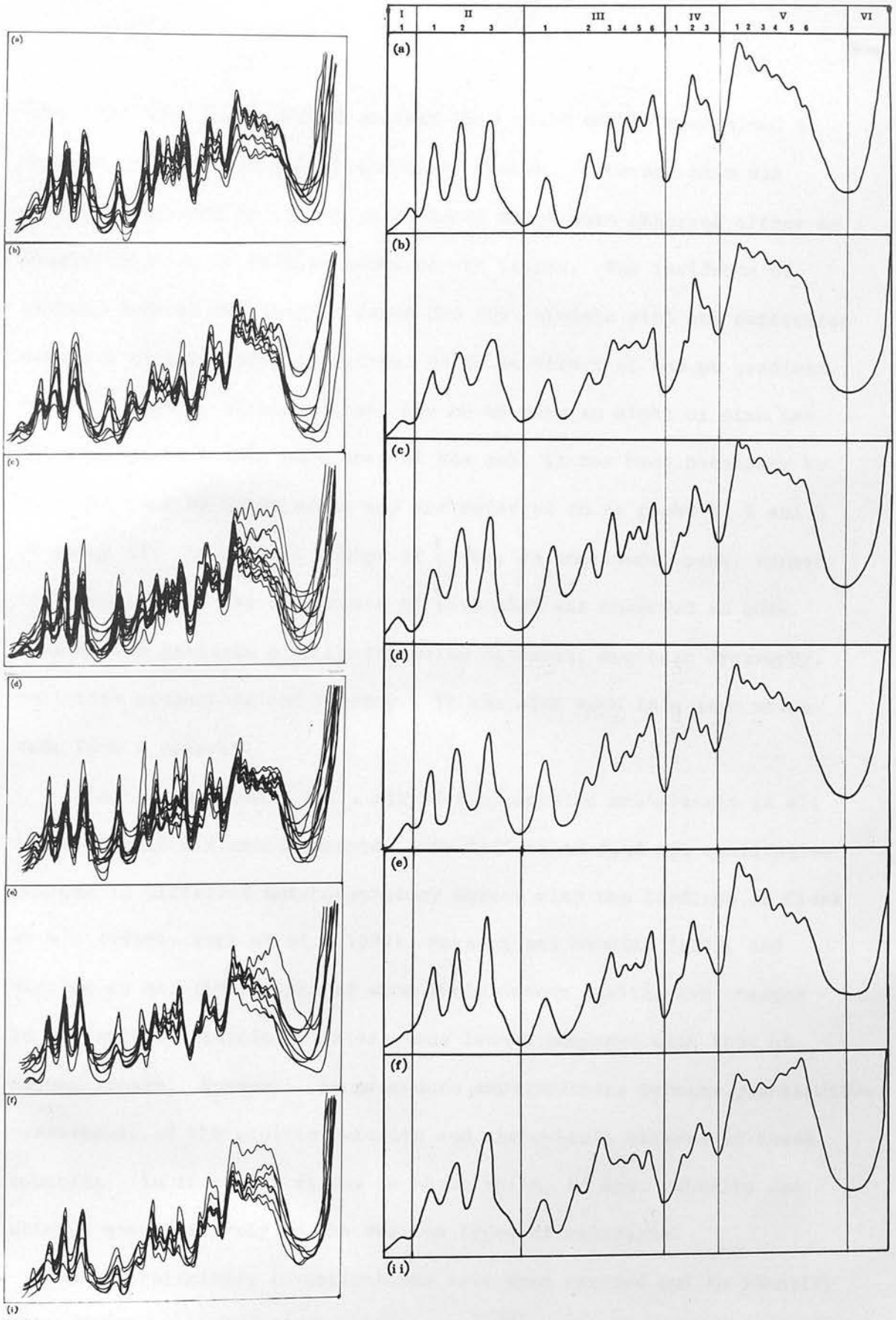


Fig. 6.6

case that some bands merged so that they could not be recognised as separate band on the gel by the densitometer. However, this was mainly restricted to the group II bands which were observed either as single, doublet or triplet peaks on the traces. The incidence of single, doublet and triplet peaks did not corelate with any particular cataract type but seemed, rather, to be an effect of the pH gradient. For this reason, although there may be as many as eight or nine individual peaks within this area of the gel, it has been necessary to consider them as three areas and are referred to as peaks 1, 2 and 3 of group II. In a small number of cases, an additional peak, termed III_o, appeared. The occurrance of this peak was observed in some lenses from patients with the following diseases; myotonic dystrophy, retinitis pigmentosa and leprosy. It was also seen in a lens which came from a cadaver.

Apart from subunit III_o, all of the proteins are present in all lenses but in different amounts. The failure to find any qualitative changes in different morphopathology agrees with the findings of Clark et al. (1969), York et al. (1972), Maraini and Mangili (1973) and Ringens et al. (1978) none of whom could detect qualitative changes in the soluble protein of cataractous lenses compared with that of normal lenses. However, the procedure employed here permits quantitative assessments of the protein subunits and the overall balance of these subunits. In this way, it may be shown which, if any, subunits are altered quantitatively in the various types of cataracts.

Some preliminary investigations have been carried out to identify the subunits in terms of crystallin nomenclature. These have involved the separation of total lens protein on ACA-54 Utrogel and electrophoresis/

electrophoresis of the resulting fractions both on S.D.S. and I.E.F. acrylamide gels.

The water soluble protein fractions from three groups of lenses - six lenses with no nuclear colour, six with yellow and six with brown - were each separated using an ACA-54 Ultrogel column and the resulting elution patterns can be seen in figure 6.7. Protein separations by Giblin and Reddy (1978) in a Sepharose 6-B column, by Jedziniak et al. (1978) on a Sephacryl S-200 Superfine gel and by Ringers et al. (1978) on Sephadex G-200 each gave similar elution patterns. A comparison with these peaks permits the identification of I to VI in figure 6.7 (Table 6.3). It can be seen from the elution patterns that the relative proportions of the crystallins

Peak	Major Crystallin Constituent
I	High Molecular Weight crystallin
II	α - crystallin
III	β_1 crystallin
IV	β_2 crystallin
V	β_3 crystallin
VI	γ - crystallin

vary with increasing nuclear colour, the first peak (I + II) and peak VI increasing while peaks IV and V decrease. No record of the cortical involvement is available for any of the lenses used and, therefore, it cannot be conclusively said that the changes observed are due to the differences in nuclear involvement only. However, the changing patterns do give some indication of the nature of changes in cataract in/

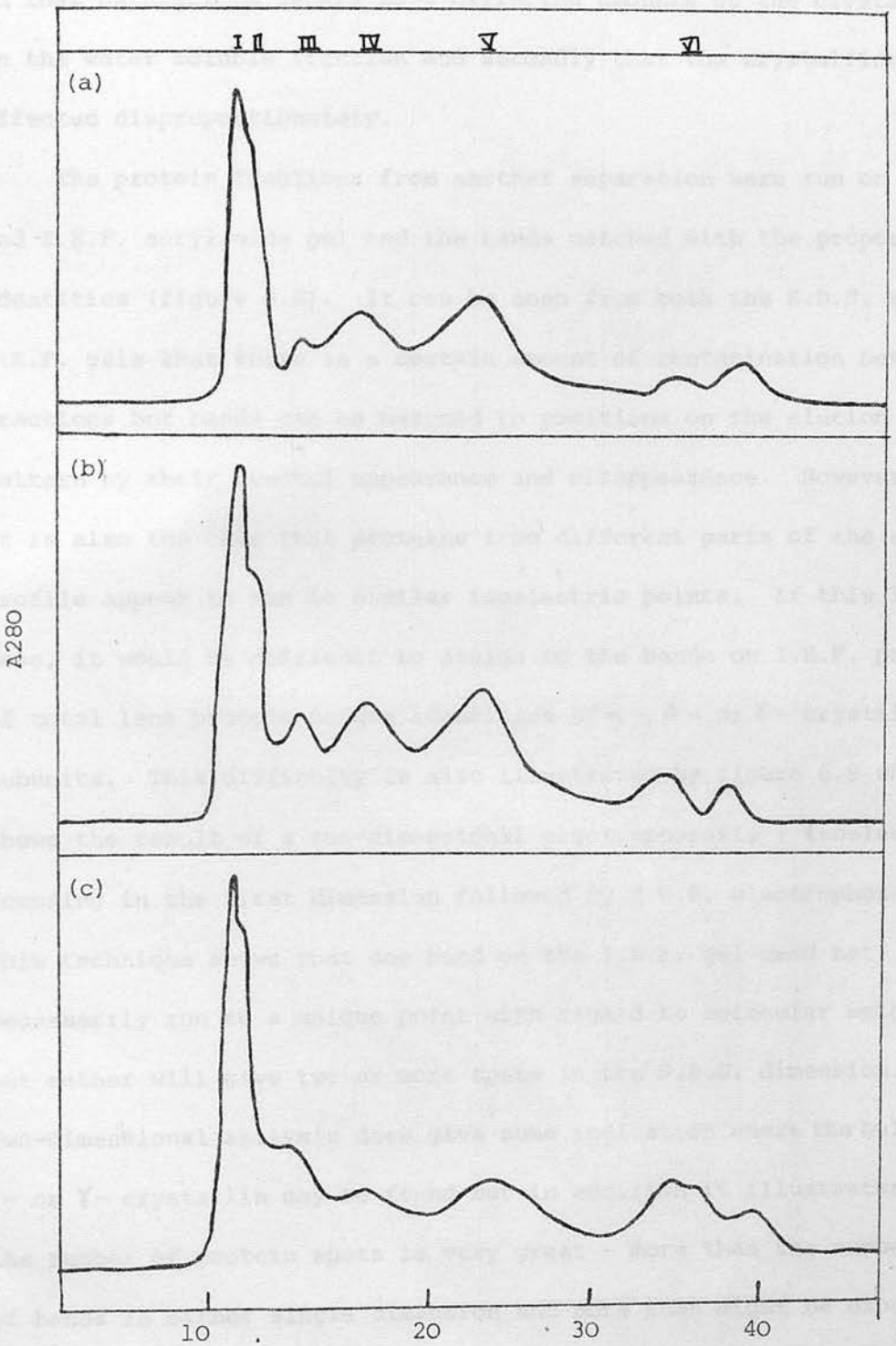


Fig. 6.7. Elution patterns of the total W.S. protein fraction of (a) cortical cataracts with no nuclear colour, (b) cortical cataracts with yellow nuclei and (c) cortical cataracts with brown nuclei. Gel chromatography was carried out on AcA54 as described in the text.

in that cataractous lenses have differing amounts of the crystallins in the water soluble fraction and secondly that the crystallins are affected disproportionately.

The protein fractions from another separation were run on S.D.S. and I.E.F. acrylamide gel and the bands matched with the proposed identities (figure 6.8). It can be seen from both the S.D.S. and I.E.F. gels that there is a certain amount of contamination between fractions but bands can be matched to positions on the elution pattern by their gradual appearance and disappearance. However, it is also the case that proteins from different parts of the elution profile appear to run to similar isoelectric points. If this is the case, it would be difficult to assign to the bands on I.E.F. profiles of total lens protein unique identities of α-, β- or γ- crystallin subunits. This difficulty is also illustrated by figure 6.9 which shows the result of a two-dimensional electrophoresis : isoelectric focusing in the first dimension followed by S.D.S. electrophoresis. This technique shows that one band on the I.E.F. gel need not necessarily run to a unique point with regard to molecular weight but rather will give two or more spots in the S.D.S. dimension. Two-dimensional analysis does give some indication where the bulk of α-β- or γ- crystallin may be found but in addition it illustrates that the number of protein spots is very great - more than the number of bands in either single dimension and more than might be expected from the number of subunits of the crystallin families suggested in the literature. This would suggest, and probably is the case as suggested by van Kleef (1974) for bovine α- crystallin in particular, that a large number of degradation and aging products are present in the/

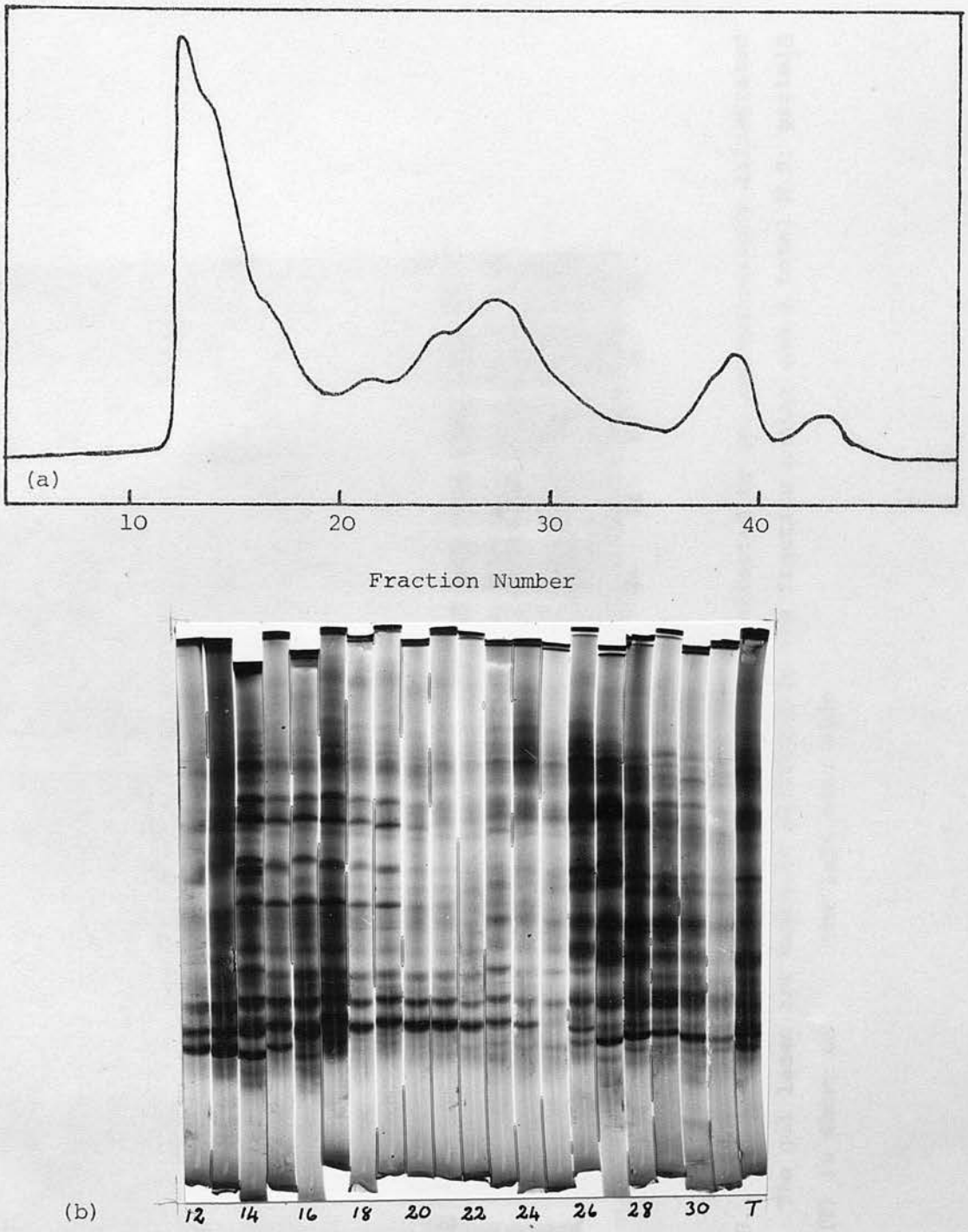


Fig.6.8. (a) Elution pattern of the total water soluble protein of cataractous lenses. The fractionation was carried out on AcA 54.
(b) Iso-electric focussing of fractions collected from gel chromotography illustrated in (a). The gels are numbered according to the fraction number and a total W.S. protein sample (T) is shown on the right hand side.
(c) (see overleaf).

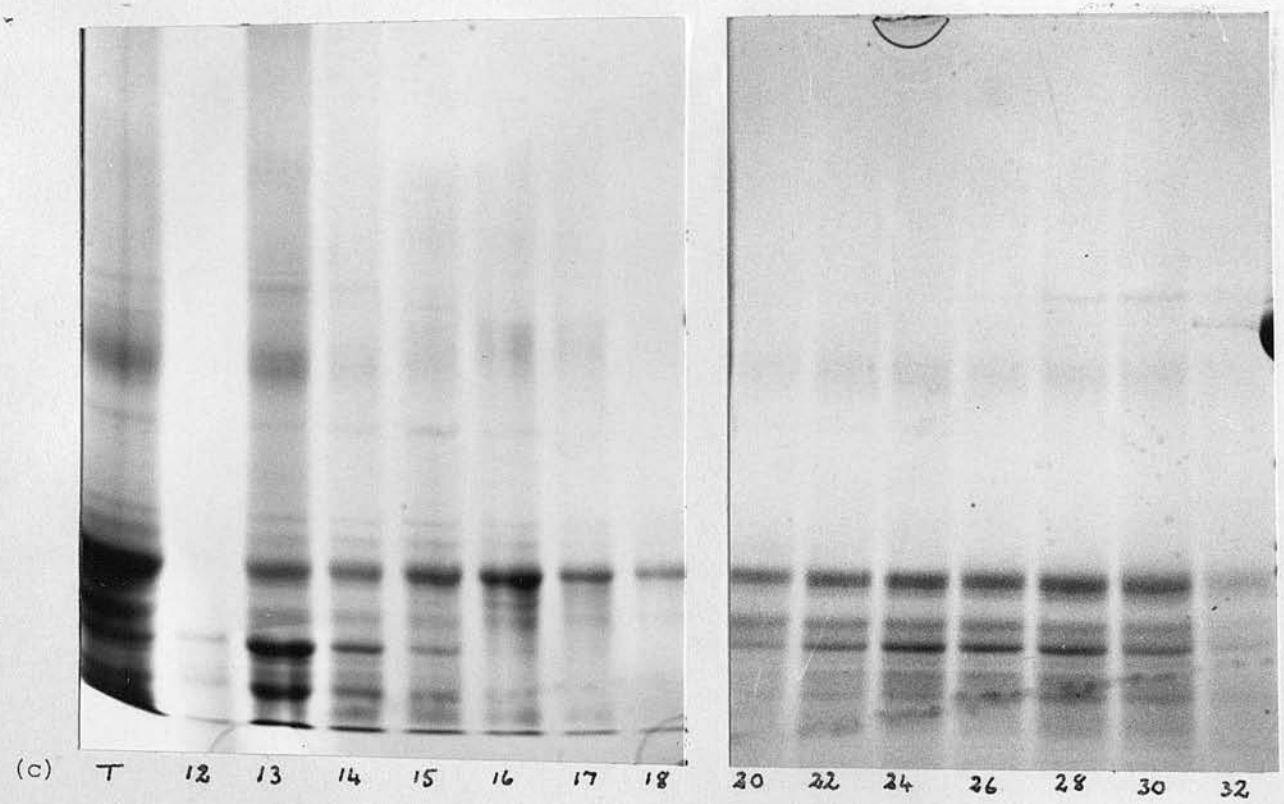
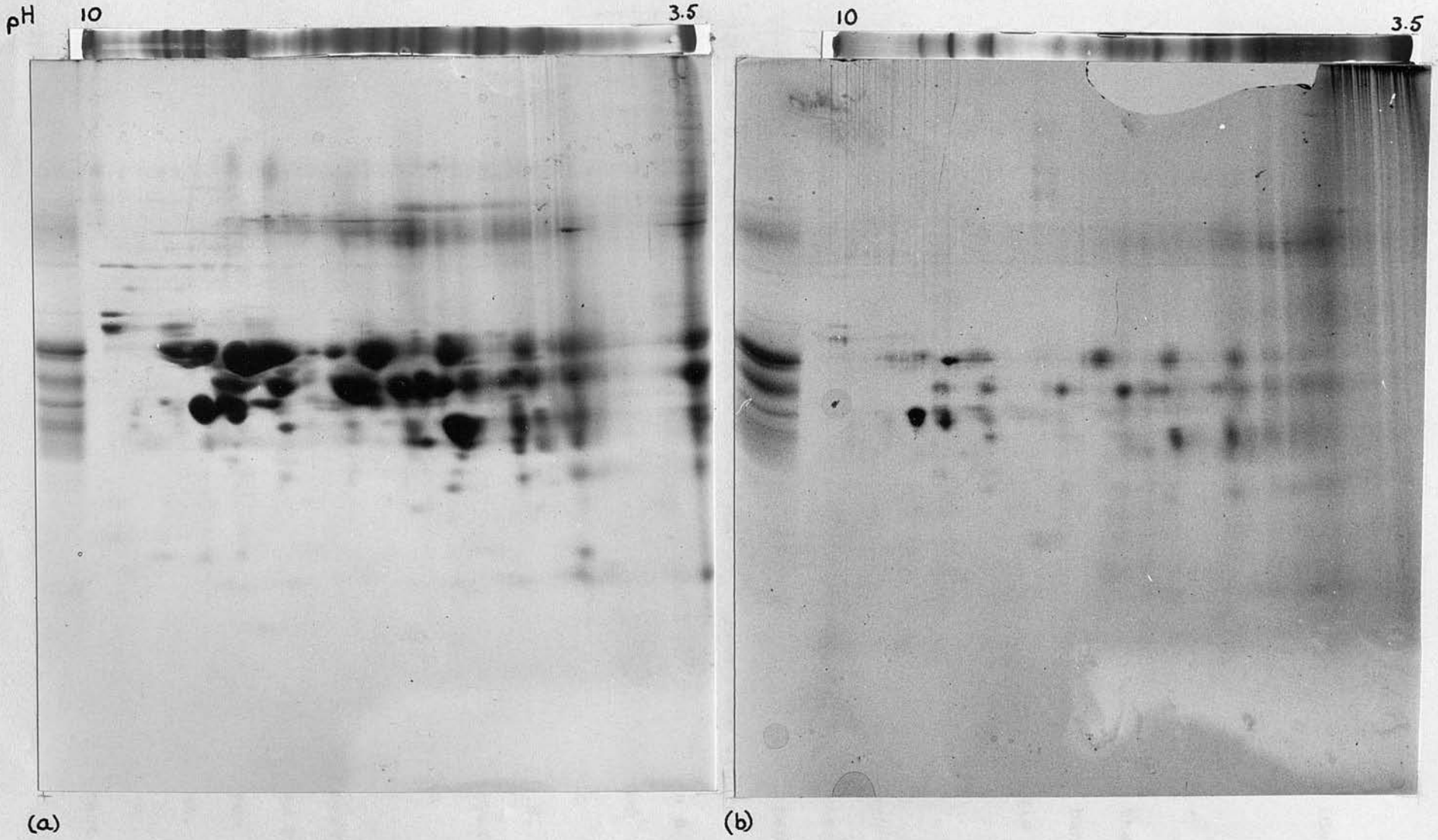


Fig. 6.8. (c) S.D.S. electrophoresis of fractions collected by gel chromatography illustrated in (a). The gel lanes are numbered according to the fraction number and a total W.S. protein sample (T) is shown on the left hand side

Fig. 6.9. Two dimensional gel electrophoresis of (a) the W.S. and (b) the U.S. protein fraction of a cataractous lens. Electrophoresis consisted of iso-electric focussing on a pH gradient of 3.5-10 in the first dimension and electrophoresis on a 15% polyacrylamide - S.D.S. gel in the second dimension. The figure illustrated that single bands at unique pI points in the I.E.F. dimension need not run to unique molecular weight positions in the S.D.S. dimension.


pH
10
3.5
10
3.5
(a)
(b)

the lens.

Although there are these ambiguities, and no immunological identification it is still possible to assign tentative identities to the peaks observed in I.E.F. suggesting the major constituent(s) (Figure 6.10). However, allowing that these bands are not representative of unique subunits, in the following sections where the protein profiles of particular cataract types are compared, the bands are given numerical identity since it is the object of this thesis to determine and describe differences in the profiles without necessarily ascribing those differences to particular proteins.

For comparison of the protein profiles and percentage values of the subunits of cataract lenses of different morphopathology, cataracts have been grouped as before and the figures presented as twenty tables (Tables 6.4 to 6.23). Tables 6.4, 6.5 and 6.6 compare the figures for the W.S. profiles of different types of cortical cataract on a non-nuclear, yellow nuclear and brown nuclear cataract background respectively and tables 6.7, 6.8 and 6.9 compare the figures for the U.S. profiles. Tables 6.10 and 6.11 compare the W.S. and U.S. profiles respectively of all cataracts of particular cortical types regardless of nuclear colour. Tables 6.12 to 6.16 each compares the figures for the W.S. profiles of similar cortical cataracts on different nuclear backgrounds and tables 6.17 to 6.21 the corresponding U.S. profiles. Tables 6.22 and 6.23 compare the W.S. and U.S. profiles respectively of all lenses of particular nuclear involvement - none, yellow or brown - regardless of cortical involvement. These tables are illustrated by figures 6.1 to 6.6. These give a visual indication of how the protein profiles vary between cataract types.

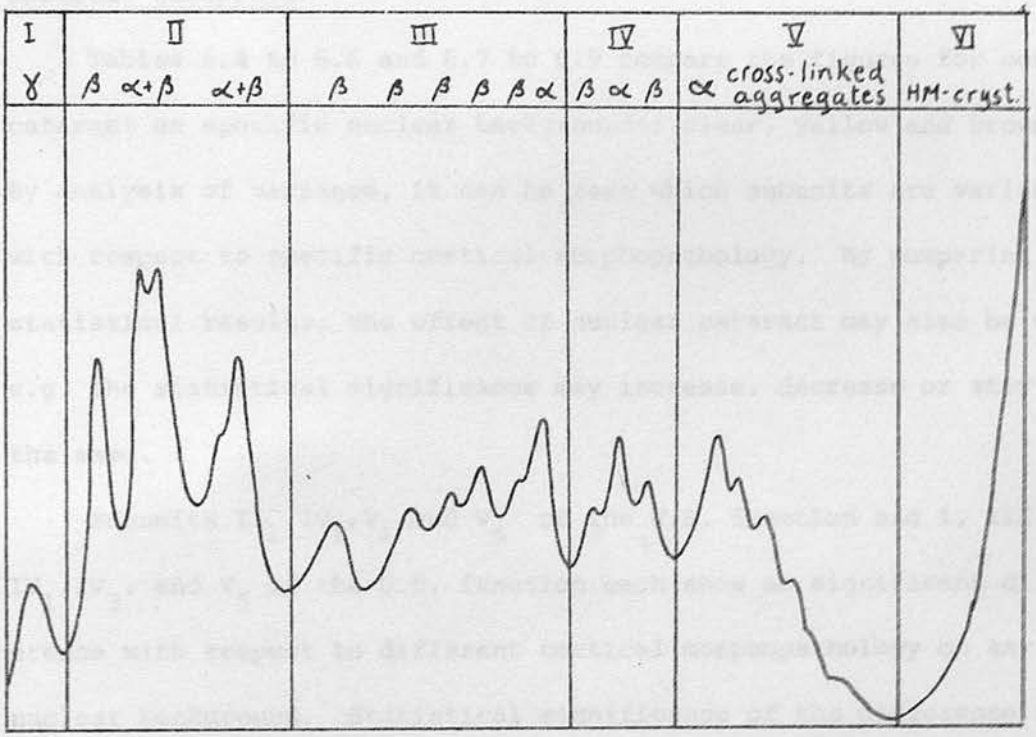


Fig. 6.10. Tentative designation, in terms of the crystallin composition, of the peaks of the gel traces, which represent the stained protein bands on I.E.F. gels. The designations are based on the data illustrated in Figures 6.8 and 6.9 only in relation to the literature and not on immunochemical tests.

types.

Cortical Cataracts.

Tables 6.4 to 6.6 and 6.7 to 6.9 compare the figures for cortical cataract on specific nuclear backgrounds; clear, yellow and brown. By analysis of variance, it can be seen which subunits are variable with respect to specific cortical morphopathology. By comparing the statistical results, the effect of nuclear cataract may also be seen e.g. the statistical significance may increase, decrease or stay the same.

Subunits II_1, IV_1, V_1 and V_5 of the W.S. fraction and I, III_5, IV_1, IV_3, and V_5 of the U.S. fraction each show no significant difference with respect to different cortical morphopathology on any nuclear background. Statistical significance of the difference in subunit II_2 decreases with increasing nuclear colour in both the W.S. and U.S. fractions; no trend is observed with increasing cortical involvement but those lenses with cuneiform plus cupuliform have the lowest percentage values for this subunit in the W.S. fractions. Subunit II_3 shows significant difference more on a clear nuclear than on a brown nuclear background but none on a yellow nuclear background but in each case, the lowest value in the more mature cortical cataract and the largest value in cupuliform cataracts.

Subunits of group III with the exception of subunit III_4 in the W.S. and III_5 in the U.S. fraction each show significant differences when on a clear background. The trend is for subunits III_1 to III_5 to decrease with increasing cortical involvement in both W.S. and U.S. fractions while the percentage value of III_6 increases with increasing cortical/

Abbreviations used in Tables 6.4 to 6.25.

IMM	immature cortical
CUN	cuneiform
CUP	cupuliform
CUN CUP	cuneiform plus cupuliform
MAT	mature cortical
HYP	hypermature cortical
YN	yellow nuclear
BN	brown nuclear

Table 6.4. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of cataracts of different cortical involvement on a clear nuclear background. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.4

W.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
IMM	0.81 (0.77)	5.73 (0.99)	10.05 (1.62)	9.69 (1.66)	0	4.03 (0.72)	7.23 (1.79)	6.55 (1.26)	3.44 (0.72)	3.25 (0.58)	7.32 (1.33)	3.12 (0.59)	8.02 (2.01)	4.20 (0.89)	7.92 (1.16)	3.38 (0.82)	2.04 (0.67)	1.31 (0.95)	0.85 (0.84)	0.43 (0.59)	8.27 (4.00)
CUN	0.72 (0.66)	5.10 (0.64)	9.26 (1.43)	8.32 (1.28)	0	4.70 (0.83)	6.68 (1.56)	5.60 (0.54)	3.43 (0.71)	2.32 (0.37)	7.94 (0.57)	2.84 (1.04)	7.62 (1.11)	3.90 (0.68)	8.25 (1.41)	4.42 (1.36)	3.50 (0.88)	3.43 (1.25)	0.53 (0.76)	0.98 (1.19)	6.62 (3.15)
CUP	0.82 (0.61)	6.10 (1.41)	10.34 (2.57)	11.08 (2.02)	0	3.80 (1.00)	7.54 (1.27)	6.46 (1.29)	3.53 (0.58)	3.27 (0.67)	8.46 (1.39)	2.80 (0.89)	8.19 (1.45)	4.42 (1.32)	7.74 (1.78)	3.73 (1.03)	2.28 (0.66)	0.25 (0.33)	0.12 (0.13)	0.12 (0.16)	8.20 (3.89)
CUN CUP	0.71 (0.55)	5.06 (1.48)	9.74 (1.38)	9.46 (0.89)	0	4.52 (1.18)	6.41 (0.99)	4.90 (1.81)	3.65 (0.56)	4.32 (0.92)	9.43 (1.02)	2.54 (0.67)	8.16 (1.89)	4.68 (1.35)	8.47 (1.13)	4.80 (0.80)	2.92 (0.83)	1.31 (1.15)	0.53 (0.50)	0.78 (0.84)	12.57 (3.54)
MAT	0.95 (0.42)	6.33 (1.70)	12.64 (3.83)	9.18 (1.64)	0	2.90 (1.67)	5.23 (1.85)	5.69 (1.53)	3.65 (0.79)	2.31 (0.75)	7.96 (1.93)	2.59 (0.62)	7.04 (1.12)	3.49 (0.95)	6.86 (0.86)	2.94 (0.65)	2.39 (0.99)	1.70 (0.97)	0.91 (0.79)	0.97 (1.08)	14.41 (5.18)
HYP	0.75 (0.60)	4.43 (0.65)	11.93 (3.53)	7.48 (0.86)	0	1.67 (1.40)	1.87 (0.51)	3.62 (1.74)	2.65 (0.62)	1.53 (0.55)	9.53 (1.97)	←	10.7 (2.12)	→	6.10 (1.56)	1.80	1.00	1.37 (1.19)	0.80 (0.75)	1.40 (1.31)	26.30 (7.60)
SIG^{n}	-	-	P<0.01	P<0.01		P<0.01	P<0.01	P<0.01	-	P<0.01	P<0.05	-	-	P<0.05	-	P=0.01	P<0.05	P<0.01	-	-	P<0.01

Table 6.5. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of cataracts of different cortical involvement on a yellow nuclear background. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.5

W.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
YN	1.80 (1.85)	5.75 (1.35)	10.67 (1.66)	9.26 (1.22)	0	4.37 (1.10)	6.31 (0.87)	5.20 (0.33)	4.00 (0.96)	2.64 (1.01)	6.88 (1.70)	2.39 (0.68)	6.68 (1.37)	4.02 (0.72)	7.42 (0.83)	3.18 (0.74)	2.48 (0.80)	2.15 (1.15)	0.78 (0.68)	0.58 (0.73)	10.72 (2.58)
YN IMM	3.18 (1.63)	5.56 (2.18)	8.99 (1.31)	9.59 (1.80)	0	4.07 (1.09)	6.43 (1.34)	5.56 (1.12)	3.63 (0.85)	2.94 (0.84)	6.87 (0.89)	2.66 (0.66)	5.46 (1.40)	4.12 (1.09)	7.12 (1.16)	3.22 (1.01)	2.36 (0.67)	2.62 (1.18)	0.94 (0.68)	2.11 (0.59)	10.71 (2.59)
YN CUN	1.31 (1.21)	6.63 (1.87)	12.39 (3.99)	9.99 (0.90)	0	3.35 (0.94)	7.61 (0.79)	5.57 (1.19)	3.41 (0.49)	2.71 (0.33)	7.63 (1.31)	2.51 (0.48)	7.75 (1.12)	3.36 (0.83)	7.47 (1.38)	3.13 (0.77)	1.45 (1.15)	1.14 (0.99)	0.45 (0.62)	0.67 (0.95)	11.07 (3.02)
YN CUP	1.02 (0.90)	6.42 (2.31)	13.40 (2.82)	10.57 (1.08)	0	4.60 (1.01)	6.93 (0.67)	7.20 (1.31)	2.77 (0.25)	2.55 (0.64)	5.07 (0.57)	2.90 (1.37)	7.95 (0.64)	3.80 (0.28)	←			16.55 (4.48)		→	9.65 (6.86)
YN CUN CUP	1.59 (1.01	6.03 (2.34)	8.93 (1.67)	9.62 (1.71)	0	3.99 (1.38)	8.31 (1.14)	5.12 (1.20)	3.74 (0.79)	3.03 (0.18)	8.32 (0.79)	2.85 (0.55)	7.34 (0.93)	4.24 (0.69)	7.62 (0.64)	4.08 (0.54)	2.70 (0.91)	3.93 (2.17)	1.09 (0.89)	1.10 (1.30)	9.02 (3.13)
YN MAT	1.30 (1.41)	4.60 (2.11)	10.83 (3.02)	8.52 (1.82)	0	5.02 (1.56)	5.38 (2.70)	4.47 (0.95)	4.40 (0.73)	2.90 (0.92)	8.17 (0.97)	2.25 (0.35)	6.83 (1.19)	2.83 (0.67)	8.03 (1.43)	4.10 (0.53)	1.47 (1.22)	1.50 (1.92)	0.90 (1.15)	2.12 (2.07)	12.68 (4.40)
SIG.^{n}	-	-	P<0.01	-		-	P<0.01	-	-	-	P<0.01	-	P<0.01	-	-	-	P=0.05	P<0.01	-	P<0.01	-

Table 6.6. Comparison of the mean percentage values ($\pm$ 1 S.D.) of the subunits of the W.S. fractions of cataracts of different cortical involvement on a brown nuclear background. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.6

W.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
BN	1.52 (0.97)	6.31 (1.00)	10.38 (2.12)	10.02 (2.63)	0	4.37 (1.05)	7.25 (1.29)	6.44 (1.21)	3.43 (0.71)	2.91 (0.66)	7.54 (1.61)	2.75 (0.81)	7.51 (1.59)	3.52 (0.74)	7.26 (1.09)	3.28 (0.66)	2.03 (0.82)	1.24 (0.98)	0.68 (0.75)	0.29 (0.42)	9.17 (3.42)
BN IMM	1.06 (1.06)	5.65 (1.41)	11.50 (3.13)	9.26 (1.44)	0	4.64 (1.49)	6.84 (1.47)	5.95 (0.80)	3.78 (0.88)	3.19 (0.43)	7.28 (0.97)	2.62 (0.51)	6.65 (0.87)	3.91 (0.83)	7.56 (0.90)	3.29 (0.89)	2.16 (0.77)	1.78 (0.70)	0.91 (0.61)	1.45 (1.21)	8.82 (2.47)
BN CUN	1.33 (1.14)	6.01 (1.70)	10.86 (2.36)	10.36 (2.19)	0	3.59 (1.35)	5.47 (1.60)	5.46 (1.02)	4.03 (0.91)	2.50 (0.82)	7.04 (1.43)	2.82 (0.65)	6.45 (1.52)	3.91 (1.21)	7.88 (1.00)	3.43 (0.84)	2.54 (0.84)	1.69 (1.36)	0.87 (0.77)	0.84 (0.86)	13.52 (4.43)
BN CUP	1.06 (0.85)	5.64 (2.11)	11.25 (3.43)	10.75 (2.31)	0	3.09 (1.66)	5.35 (1.52)	5.37 (1.74)	3.93 (1.25)	2.91 (0.82)	8.03 (1.96)	2.46 (0.86)	7.86 (1.35)	3.81 (0.83)	7.99 (1.56)	3.41 (1.22)	1.44 (0.80)	1.39 (1.41)	0.55 (0.72)	0.39 (0.66)	10.21 (3.90)
BN CUP CUN	2.44 (1.81)	6.19 (1.58)	9.12 (2.01)	9.58 (1.69)	0	3.77 (0.67)	6.13 (1.03)	5.58 (0.74)	3.58 (0.52)	2.36 (0.78)	7.96 (1.43)	2.51 (0.59)	7.62 (1.16)	4.07 (0.63)	6.76 (1.29)	3.85 (0.52)	2.72 (0.83)	1.79 (1.65)	0.88 (0.99)	0.99 (1.35)	9.79 (3.16)
BN MAT	1.48 (1.27)	5.02 (1.69)	10.35 (2.88)	8.91 (2.35)	0	3.02 (1.64)	4.13 (2.01)	5.67 (2.05)	3.33 (0.79)	2.53 (1.16)	8.64 (2.16)	2.65 (1.09)	7.08 (1.40)	4.04 (1.35)	7.12 (1.50)	3.76 (1.05)	2.48 (0.95)	1.78 (0.89)	1.03 (0.85)	1.30 (1.26)	12.01 (5.04)
SIG^n	$P<0.01$	-	-	$P<0.05$		$P<0.05$	$P<0.05$	-	$P<0.05$	-	-	-	-	-	-	-	$P<0.01$	-	-	$P<0.01$	$P<0.01$

Table 6.7. Comparison of the mean percentage values ($\pm$ 1 S.D.) of the subunits of the U.S. fractions of cataracts of different cortical involvement on a clear nuclear background. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.7

U.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
IMM	0.62 (0.83)	2.15 (0.74)	4.33 (1.07)	4.79 (1.73)	0	2.72 (0.96)	3.06 (0.98)	4.55 (1.03)	3.04 (0.53)	2.84 (0.81)	4.87 (0.87)	2.92 (0.30)	5.84 (1.05)	3.80 (0.68)	9.26 (1.51)	5.22 (1.05)	6.14 (1.58)	6.17 (1.37)	3.30 (0.91)	5.61 (1.99)	14.39 (3.63)
CUN	0.02 (0.04)	2.59 (0.30)	3.30 (0.32)	5.64 (1.38)	0	1.22 (0.55)	1.06 (0.50)	3.78 (1.22)	2.44 (0.34)	2.64 (0.66)	4.77 (0.84)	2.85 (1.02)	6.35 (0.51)	4.24 (0.42)	9.26 (0.78)	5.32 (0.43)	6.30 (0.51)	6.68 (0.61)	4.64 (0.72)	9.58 (1.48)	17.28 (4.11)
CUP	0.57 (0.41)	3.83 (0.98)	4.73 (1.64)	4.83 (1.51)	0	3.01 (0.90)	2.96 (1.45)	3.81 (1.71)	2.40 (1.31)	2.87 (1.23)	3.76 (1.33)	2.48 (0.80)	5.32 (0.89)	3.71 (0.85)	8.78 (0.73)	5.41 (0.93)	6.45 (1.38)	5.94 (1.03)	3.68 (1.16)	5.71 (1.62)	14.90 (6.68)
CUN CUP	0.10 (0.19)	1.57 (0.30)	3.22 (0.58)	4.43 (0.87)	0	1.18 (0.68)	1.24 (0.99)	2.87 (0.95)	1.96 (0.68)	2.05 (0.97)	5.59 (1.03)	2.47 (0.64)	5.57 (0.93)	4.29 (0.35)	9.60 (0.57)	6.59 (1.10)	5.64 (1.38)	6.45 (0.46)	3.84 (0.92)	8.39 (5.33)	18.81 (6.17)
MAT	0.33 (0.46)	1.76 (1.30)	2.82 (1.37)	4.20 (1.69)	0	2.12 (1.46)	2.71 (1.14)	3.55 (1.48)	2.51 (0.68)	2.45 (0.80)	4.12 (1.20)	2.95 (0.72)	6.11 (0.89)	3.58 (0.77)	9.37 (1.32)	5.45 (1.42)	6.01 (1.01)	6.73 (1.40)	4.07 (0.97)	10.88 (4.65)	17.45 (5.18)
HYP	0.32 (0.27)	0.57 (0.38)	2.59 (1.55)	1.58 (0.47)	0	1.40 (0.49)	1.05 (0.86)	1.66 (0.25)	2.01 (0.78)	2.18 (1.28)	4.47 (1.38)	←	9.02 (2.49)	→ 3.68 (1.12)	9.79 (1.78)	6.43 (1.78)	6.76 (1.73)	8.32 (2.78)	5.72 (1.56)	10.05 (4.85)	19.97 (9.18)
SIG^n	-	P<0.01	P<0.01	P<0.01		P<0.01	P<0.01	P<0.01	P<0.05	-	P<0.05	-	-	-	-	-	-	-	P<0.01	P<0.01	-

Table 6.8. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of cataracts of different cortical involvement on a yellow nuclear background. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.8

U.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
YN	0.36 (0.51)	3.43 (1.19)	4.77 (0.74)	5.25 (1.31)	0	1.84 (0.80)	2.33 (0.53)	4.56 (0.94)	2.86 (0.71)	2.43 (0.53)	4.40 (1.21)	3.04 (0.49)	4.63 (0.81)	4.11 (0.53)	8.17 (0.78)	5.35 (1.03)	5.35 (0.93)	6.27 (0.79)	3.41 (0.86)	7.26 (1.76)	16.62 (2.69)
YN IMM	0.27 (0.32)	2.64 (1.04)	3.89 (0.78)	5.45 (1.31)	0	2.34 (0.95)	3.19 (1.43)	4.42 (1.21)	2.89 (0.46)	2.28 (0.71)	5.14 (0.78)	2.86 (0.53)	4.96 (0.68)	3.82 (0.84)	8.18 (0.81)	4.72 (0.89)	5.21 (0.83)	5.61 (0.89)	3.49 (0.72)	7.44 (1.11)	18.08 (4.41)
YN CUN	0.33 (0.41)	1.88 (0.95)	3.37 (1.08)	4.26 (1.31)	0	1.74 (1.17)	1.76 (0.89)	3.58 (1.12)	2.88 (0.47)	2.73 (0.82)	4.24 (1.03)	2.32 (0.59)	6.74 (0.79)	4.23 (0.68)	10.73 (1.49)	6.52 (1.27)	5.27 (1.03)	5.83 (1.00)	3.44 (1.22)	4.67 (1.78)	18.22 (5.23)
YN CUP	0.13 (0.15)	2.83 (0.71)	3.30 (1.91)	3.53 (1.18)	0	1.87 (0.93)	1.27 (0.72)	4.17 (0.64)	2.57 (0.72)	1.83 (0.40)	3.98 (1.51)		8.85 (0.55)	3.92 (0.22)	9.22 (1.48)	5.60 (0.44)	6.63 (0.40)	7.93 (0.78)	4.52 (0.74)	13.93 (1.86)	15.10 (3.71)
YN CUN CUP	0.48 (0.42)	2.16 (0.70)	4.21 (0.99)	4.90 (1.01)	0	1.49 (1.11)	2.86 (0.41)	3.69 (0.84)	2.48 (0.65)	1.90 (0.46)	4.92 (0.44)	2.56 (1.12)	5.44 (1.20)	3.79 (0.63)	8.47 (1.35)	5.35 (1.19)	5.59 (0.66)	5.33 (0.57)	3.06 (0.70)	5.63 (2.41)	18.11 (4.41)
YN MAT	0.30 (0.08)	2.02 (1.34)	2.60 (1.05)	3.82 (1.35)	0	3.07 (0.57)	1.98 (1.63)	4.93 (0.87)	2.08 (1.00)	2.65 (1.10)	4.90 (0.98)	2.97 (0.93)	6.23 (1.24)	4.63 (0.57)	9.50 (0.50)	5.27 (0.50)	5.53 (0.06)	6.07 (0.51)	4.25 (0.73)	10.12 (0.90)	12.43 (1.20)
SIG^n	-	P<0.05	P<0.05	P<0.05		-	P<0.01	-	-	-	-	-	P<0.01	-	P<0.01	P<0.01	-	P<0.01	-	P<0.01	P<0.01

Table 6.9. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of cataracts of different cortical involvement on a brown nuclear background. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.9

U.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
BN	0.56 (0.61)	2.29 (1.36)	4.16 (1.38)	4.92 (1.44)	0	2.41 (0.90)	2.67 (1.21)	4.06 (1.60)	2.62 (0.72)	2.59 (1.13)	4.28 (1.04)	3.04 (0.50)	6.35 (1.04)	4.35 (0.63)	9.59 (1.76)	5.17 (1.42)	6.16 (1.44)	6.22 (1.19)	4.13 (1.41)	9.97 (3.94)	15.43 (2.43)
BN IMM	0.33 (0.36)	2.49 (1.50)	3.21 (1.60)	4.58 (2.17)	0	1.99 (1.19)	2.73 (0.94)	4.01 (1.50)	2.42 (0.54)	2.09 (0.59)	4.82 (1.49)	2.89 (0.77)	5.40 (0.98)	3.88 (0.69)	9.48 (1.34)	5.16 (1.07)	5.70 (1.25)	5.43 (1.64)	4.24 (1.20)	10.49 (2.33)	18.14 (5.66)
BN CUN	0.30 (0.47)	2.31 (1.32)	3.94 (1.36)	4.33 (1.43)	0	2.51 (0.77)	2.91 (0.99)	3.76 (1.22)	2.37 (0.68)	2.20 (0.65)	4.43 (1.18)	2.84 (0.65)	5.50 (1.28)	3.82 (1.18)	8.95 (1.35)	5.12 (1.05)	2.79 (0.95)	6.14 (0.91)	4.11 (0.93)	13.70 (3.76)	16.68 (3.17)
BN CUP	0.41 (0.57)	2.07 (1.46)	3.42 (1.35)	4.19 (1.59)	0	1.63 (1.23)	1.44 (1.28)	3.14 (1.68)	2.45 (0.75)	2.62 (1.34)	3.67 (1.66)	2.54 (1.08)	5.87 (1.59)	3.50 (0.98)	9.65 (1.83)	5.21 (0.89)	5.91 (1.02)	5.91 (1.06)	3.51 (0.76)	9.08 (3.40)	15.59 (4.82)
BN CUN CUP	0.29 (0.36)	2.04 (0.95)	3.44 (1.28)	3.94 (1.18)	0	1.61 (0.83)	1.57 (1.33)	2.14 (0.89)	2.04 (0.66)	2.38 (0.66)	4.41 (0.99)	2.43 (0.80)	5.58 (1.08)	3.87 (0.86)	9.34 (1.05)	5.74 (1.49)	6.20 (0.89)	6.23 (0.92)	4.90 (0.97)	16.36 (4.42)	17.11 (4.76)
BN MAT	0.43 (0.55)	1.67 (0.96)	3.09 (1.31)	3.74 (1.41)	0	1.76 (1.09)	2.37 (1.16)	3.41 (1.25)	2.38 (0.74)	2.36 (0.84)	4.17 (0.94)	2.78 (0.73)	5.74 (1.04)	3.98 (0.81)	8.83 (1.30)	5.67 (1.13)	6.11 (0.80)	6.46 (1.28)	4.47 (0.93)	11.67 (4.07)	15.42 (4.66)
SIG^n	-	-	-	-		P<0.01	P<0.01	P<0.01	-	-	-	-	-	-	-	-	-	-	P<0.01	P<0.01	-

Table 6.10. Comparison of the mean percentage values ($\pm$ 1 S.D.) of the subunits of the W.S. fractions of cataracts of different cortical involvement regardless of nuclear colour. Analysis of variance is carried out to determine the statistical significant difference.

Table 11. Comparison of the mean percentage values ($\pm$ 1 S.D.) of the subunits of the U.S. fractions of cataracts of different cortical involvement regardless of nuclear colour. Analysis of variance is carried out to determine the statistical significant difference.

TABLE 6.10

W.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
IMM	1.75 (1.62)	6.01 (0.82)	10.08 (2.29)	9.53 (1.70)	0	4.23 (1.16)	6.81 (1.54)	5.96 (1.16)	3.62 (0.81)	3.10 (0.67)	7.11 (1.07)	2.78 (0.63)	6.51 (1.82)	4.09 (1.01)	7.45 (1.11)	3.29 (0.90)	2.21 (0.69)	1.99 (0.76)	0.91 (0.70)	1.42 (1.06)	9.39 (3.23)
CUN	1.26 (1.11)	6.11 (1.74)	11.21 (3.05)	10.05 (1.87)	0	3.61 (1.23)	6.23 (1.68)	5.51 (1.02)	3.74 (0.81)	2.56 (0.66)	7.35 (1.34)	2.72 (0.65)	7.01 (1.47)	3.74 (1.06)	7.83 (1.15)	3.51 (0.96)	2.36 (1.11)	1.62 (1.34)	0.69 (0.73)	0.79 (0.91)	11.04 (4.41)
CUP	0.99 (0.78)	5.86 (1.93)	11.21 (3.18)	10.82 (2.12)	0	3.44 (1.49)	6.09 (1.70)	5.82 (1.69)	3.72 (1.09)	3.02 (0.77)	7.90 (1.91)	2.62 (0.92)	7.98 (1.32)	4.02 (0.91)	7.90 (1.66)	3.83 (1.26)	1.74 (0.84)	1.04 (1.29)	0.42 (0.63)	0.30 (0.57)	9.59 (4.23)
CUN CUP	1.84 (1.58)	5.88 (1.75)	9.21 (1.78)	9.56 (1.51)	0	4.01 (1.01)	6.66 (1.34)	5.26 (1.24)	3.64 (0.60)	2.96 (1.04)	8.39 (1.34)	2.58 (0.60)	7.72 (1.35)	4.26 (0.89)	7.32 (1.33)	4.10 (0.67)	2.76 (0.81)	2.00 (1.78)	0.93 (0.86)	0.96 (0.98)	10.16 (3.38)
MAT	1.20 (1.12)	5.68 (1.83)	11.63 (3.41)	9.02 (1.97)	0	3.08 (1.70)	4.79 (2.02)	5.61 (1.76)	3.51 (0.93)	2.43 (0.95)	8.23 (1.98)	2.60 (0.86)	7.04 (1.24)	3.68 (1.15)	7.10 (1.28)	3.27 (1.31)	2.35 (1.00)	1.72 (0.97)	0.96 (0.85)	1.18 (1.24)	13.35 (4.14)
SIG^n	P<0.05	-	P<0.01	P<0.01		P<0.01	P<0.01	-	-	P<0.01	P<0.01	-	P<0.01	-	-	P<0.05	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01

TABLE 6.11

U.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
IMM	0.40 (0.56)	2.45 (1.18)	3.77 (1.29)	4.92 (1.79)	0	2.32 (1.06)	2.99 (1.15)	4.32 (1.26)	2.77 (0.59)	2.40 (0.76)	4.92 (1.11)	2.89 (0.55)	5.44 (0.97)	3.83 (0.73)	9.00 (1.35)	5.01 (1.00)	5.66 (1.26)	5.70 (1.35)	3.68 (1.02)	7.96 (2.73)	17.10 (4.91)
CUN	0.28 (0.43)	2.22 (1.13)	3.69 (1.22)	4.46 (1.43)	0	2.08 (1.01)	2.28 (1.13)	3.71 (1.16)	2.53 (0.62)	2.41 (0.73)	4.52 (0.84)	2.70 (0.71)	5.98 (1.01)	4.00 (0.98)	9.54 (1.55)	5.59 (1.24)	5.75 (0.98)	6.09 (0.93)	3.92 (11.09)	10.27 (5.04)	17.30 (4.09)
CUP	0.44 (0.51)	2.59 (1.49)	3.77 (1.55)	4.31 (1.55)	0	1.98 (1.26)	1.83 (1.44)	3.42 (1.64)	2.44 (0.93)	2.63 (1.26)	3.73 (1.53)	2.52 (0.97)	5.66 (1.37)	3.61 '0.88)	9.36 (1.57)	5.31 '0.86)	6.14 '1.13)	6.13 (1.18)	3.70 (0.93)	8.69 (3.65)	15.28 (4.53)
CUN CUP	0.29 (0.36)	1.95 (0.80)	3.57 (1.13)	4.31 (1.11)	0	1.47 (0.87)	1.76 (1.27)	2.71 '1.08)	2.12 (0.68)	2.20 (0.73)	4.10 (2.76)	2.47 (0.80)	5.62 (1.03)	3.96 (0.71)	8.58 (2.59)	5.86 (1.38)	5.89 (1.02)	6.10 (0.84)	4.33 (1.15)	11.50 (6.36)	17.78 (5.06)
MAT	0.37 (0.49)	1.74 (1.17)	2.93 (1.33)	3.97 (1.55)	0	2.00 (1.31)	2.51 (1.17)	3.54 (1.38)	2.43 (0.70)	2.42 (0.83)	4.18 (1.09)	2.87 (2.18)	5.94 (0.98)	3.81 (0.82)	9.16 (1.30)	5.54 (1.27)	6.03 (0.90)	6.57 (1.32)	4.28 (0.95)	11.20 (4.27)	16.50 (4.77)
SIG^n	-	P<0.01	P<0.01	P<0.05		P<0.05	P<0.01	P<0.01	P<0.01	-	P<0.01	-	-	-	-	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	-

cortical involvement in the W.S. fraction and is high in the U.S. fraction of lenses with immature and cuneiform plus cupuliform cortical involvement. These trends can be observed on yellow and brown nuclear backgrounds also although, in the W.S. fraction only subunits III_2 and III_6 on a yellow background and subunits III_1, III_2 and III_4 on a brown background show significant difference. In the U.S. fraction, III_2 on a yellow background and III_1, III_2 and III_3 on a brown background show statistically significant differences. Thus, only subunit III_2 shows significant differences on all nuclear backgrounds in both the W.S. and V.S. fractions.

Little variability is seen in group IV subunits, the only significant differences being observed in IV_2 on a yellow background in both the W.S. and U.S. fractions and in IV_3 in a clear nuclear background in the W.S. fraction only. In the W.S. fraction the highest percentage value of IV_2 is found in lenses with cupuliform opacities in each case.

In group V in the water soluble fraction higher percentage values are seen for all subunits, V_1 to V_6 in those lenses with cuneiform opacities, including cupuliform plus cuneiform opacities, when those lenses with no nuclear involvement are considered; this difference is significant in subunits V_2, V_3 and V_4 particularly. To a certain extent this general observation is true on a brown nuclear background while on a yellow nuclear background the opposite is true; these subunits show low percentage values. In lenses with either clear or brown nuclei, low percentage values are seen in lenses with cupuliform opacities and this is probably the case also in those lenses with/

with yellow nuclei but in which, the protein bands cannot be distinguished so that individual peak values can be obtained. The total value (16.55%), however is lower than in the cataractous types except those with yellow nucleus plus cuneiform cataract. Statistically significant differences are found between each of the subunits V_3, V_4 and V_6 in lenses with a yellow nucleus and V_3 and V_6 in lenses with a brown nucleus. Thus, subunit V_3 shows significant difference in different cortical morphopathologies on each type of nuclear background.

With regard to the U.S. fraction, the situation is somewhat reversed. In lenses with cuneiform involvement on clear and brown nuclear backgrounds, lower percentage values are observed for subunits V_1, to V_6 with the exception of V_6 lenses with a brown nuclei ; statistically significant differences are seen in subunits V_5 and V_6 in each case. In the case of lenses with a yellow nucleus, higher percentage values are observed in lenses with cuneiform opacities although in some cases, subunits V_3 to V_6 higher values are observed in lenses with cupuliform involvement, statistically significant differences are seen in subunits V_1, V_2, V_4 and V_6. In contrast to the W.S. fraction, subunit V_5 is the only subunit not to show significant differences on any nuclear background. Subunit V_6 shows significant differences in all three cases with an apparent trend of increasing value in cuneiform and more mature cortical cataracts while cupuliform cataracts show lower values. In the case of yellow nuclear background, however the value is lower in cuneiform and higher in cupuliform. It may cautiously be said that this trend is the case in/

in the W.S. fraction also.

The situation regarding subunit VI_1, which is the heavy molecular weight protein which does not enter the gel rather than a subunit with an isoelectric point near pH3.5, is rather similar to subunit V_6. It appears to increase in value with increasing cortical involvement with lower values in lenses with cupuliform involvement in both the W.S. and U.S. fractions. Statistically significant differences are observed between lenses with clear nuclei and also between those with brown nuclei in the W.S. fraction and between lenses with a yellow nucleus in the U.S. fraction.

Tables 6.10 and 6.11 compare the W.S. and U.S. fractions respectively of all lenses of particular cortical involvement regardless of nuclear colour. The degree of variability as observed in tables 6.4 to 6.6 and tables 6.7 to 6.9 is endorsed.

Nuclear Cataracts.

Tables 6.12 to 6.16 and 6.17 to 6.21 compare the subunit percentage values in the W.S. and U.S. fractions respectively of cataracts of similar cortical morphopathology but differing nuclear colour. Again, analysis of variance is used to find which subunits show significant difference with respect to nucleur colour and whether this difference is consistent irrespective of cortical involvement.

In the W.S. fraction, the percentage value of subunit I_1 tends to increase with increasing nuclear colour but statistical significance is observed on a cuneiform plus cupuliform background. In the case of immature cortical involvement, those lenses with a yellow nucleus have a statistically significant larger value. Nothing similar is observed in the U.S. fraction.

Little/

Table 6.12. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of immature cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.13. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of cuneiform cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.14. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of cupuliform cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.15. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of cuneiform plus cupuliform cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.16. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the W.S. fractions of mature cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

TABLE 6.12

W.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
IMM	0.81 (0.77)	5.73 (0.99)	10.05 (1.62)	9.69 (1.66)	0	4.03 (0.72)	7.23 (1.79)	6.55 (1.26)	3.44 (0.72)	3.25 (0.58)	7.32 (1.33)	3.12 (0.59)	8.02 (2.01)	4.20 (0.89)	7.92 (1.16)	3.38 (0.82)	2.04 (0.67)	1.31 (0.95)	0.85 (0.84)	0.43 (0.59)	8.27 (4.00)
YN IMM	3.18 (1.63)	5.56 (2.18)	8.99 (1.31)	9.59 (1.80)	0	4.07 (1.09)	6.43 (1.34)	5.56 (1.12)	3.63 (0.85)	2.94 (0.84)	6.87 (0.89)	2.66 (0.66)	5.46 (1.40)	4.12 (1.09)	7.12 (1.16)	3.22 (1.01)	2.36 (0.67)	2.62 (1.18)	0.94 (0.68)	2.11 (0.59)	10.71 (2.59)
BN IMM	1.06 (1.06)	5.65 (1.41)	11.50 (3.13)	9.26 (1.44)	0	4.64 (1.49)	6.84 (1.47)	5.95 (0.80)	3.78 (0.88)	3.19 (0.43)	7.28 (0.97)	2.62 (0.51)	6.65 (0.87)	3.91 (0.83)	7.46 (0.90)	3.29 (0.89)	2.16 (0.77)	1.78 (0.70)	0.91 (0.61)	1.45 (1.21)	8.82 (2.47)
	P<0.01	NS	P<0.01	NS		NS	NS	P<0.05	NS	NS	NS	NS	P<0.01	NS	NS	NS	NS	P<0.01	NS	P<0.01	NS

TABLE 6.13

CUN	0.72 (0.66)	5.10 (0.64)	9.26 (1.43)	8.32 (1.28)	0	4.70 (0.83)	6.68 (1.56)	5.60 (0.54)	3.43 (0.71)	2.32 (0.37)	7.94 (0.57)	2.84 (1.04)	7.62 (1.11)	3.90 (0.68)	8.25 (1.41)	4.42 (1.36)	3.50 (0.88)	3.43 (1.25)	0.53 (0.76)	0.98 (1.19)	6.62 (3.15)
YN CUN	1.31 (1.21)	6.63 (1.87)	12.39 (3.99)	9.99 (0.90)	0	3.35 (0.94)	7.61 (0.79)	5.57 (1.19)	3.41 (0.49)	2.71 (0.33)	7.63 (1.31)	2.51 (0.48)	7.75 (1.12)	3.36 (0.83)	7.47 (1.38)	3.13 (0.77)	1.45 (1.15)	1.14 (0.99)	0.45 (0.62)	0.67 (0.95)	11.07 (3.02)
BN CUN	1.33 (1.14)	6.01 (1.70)	10.86 (2.36)	10.36 (2.19)	0	3.59 (1.35)	5.47 (1.60)	5.46 (1.02)	4.03 (0.91)	2.50 (0.82)	7.04 (1.43)	2.82 (0.65)	6.45 (1.52)	3.91 (1.21)	7.88 (1.00)	3.43 (0.84)	2.54 (0.84)	1.69 (1.36)	0.87 (0.77)	0.84 (0.86)	13.52 (4.43)
	NS	NS	NS	NS		NS	P<0.01	NS	P<0.05	NS	NS	NS	P<0.05	NS	NS	NS	P<0.01	P<0.05	NS	NS	P<0.01

TABLE 6.14

CUP	0.82 (0.61)	6.10 (1.41)	10.34 (2.57)	11.08 (2.02)	0	3.80 (1.00)	7.54 (1.27)	6.46 (0.58)	3.53 (0.67)	3.27 (1.39)	8.46 (1.39)	2.80 (0.89)	8.19 (1.45)	4.42 (1.32)	7.74 (1.78)	3.73 (1.03)	2.28 (0.66)	0.25 (0.33)	0.12 (0.13)	0.12 (0.16)	8.20 (3.89)
YN CUP	1.02 (0.90)	6.42 (2.31)	13.40 (2.82)	10.57 (1.08)	0	4.60 (1.01)	6.93 (0.67)	7.20 (1.31)	2.77 (0.25)	2.55 (0.64)	5.07 (0.57)	2.90 (1.37)	7.95 (0.64)	3.80 (0.28)	←			16.55 (4.48)		→	9.65 (6.86)
BN CUP	1.06 (0.85)	5.64 (2.11)	11.25 (3.43)	10.75 (2.31)	0	3.09 (1.66)	5.35 (1.52)	5.37 (1.74)	3.93 (1.25)	2.91 (0.82)	8.03 (1.96)	2.46 (0.86)	7.86 (1.35)	3.81 (0.83)	7.99 (1.56)	3.41 (1.22)	1.44 (0.80)	1.39 (1.41)	0.55 (0.72)	0.39 (0.66)	10.21 (3.90)
	NS	NS	NS	NS		NS	P<0.01	NS	NS	NS	P<0.05	NS	NS	NS	NS	NS	P<0.05	NS	NS	NS	NS

TABLE 6.15

CUN CUP	0.71 (0.55)	5.06 (1.48)	9.74 (1.38)	9.46 (0.89)	0	4.52 (1.18)	6.41 (0.99)	4.90 (1.81)	3.65 (0.56)	4.32 (0.92)	9.43 (1.02)	2.54 (0.67)	8.16 (1.89)	4.68 (1.35)	8.47 (1.13)	4.80 (0.80)	2.92 (0.83)	1.31 (1.15)	0.53 (0.50)	0.78 (0.84)	12.57 (3.54)
YN CUN CUP	1.59 (1.01)	6.03 (2.34)	8.93 (1.67)	9.62 (1.71)	0	3.99 (1.38)	8.31 (1.14)	5.12 (1.20)	3.74 (0.79)	3.03 (0.18)	8.32 (0.79)	2.85 (0.55)	7.34 (0.93)	4.24 (0.69)	7.62 (0.64)	4.08 (0.54)	2.70 (0.91)	9.93 (2.17)	1.09 (0.89)	1.10 (1.30)	9.02 (3.13)
BN CUN CUP	2.44 (1.81)	6.19 (1.58)	9.12 (2.01)	9.58 (1.69)	0	3.77 (0.67)	6.13 (1.03)	5.58 (0.74)	3.58 (0.52)	2.36 (0.78)	7.96 (1.43)	2.51 (0.59)	7.62 (1.16)	4.07 (0.63)	6.76 (1.29)	3.85 (0.52)	2.72 (0.83)	1.79 (1.65)	0.88 (0.99)	0.99 (1.35)	9.79 (3.16)
	P<0.05	NS	NS	NS		NS	P<0.01	NS	NS	P<0.01	P<0.05	NS	NS	NS	P<0.05	P<0.05	NS	P<0.01	NS	P<0.05	NS

TABLE 6.16

MAT	0.95 (0.42)	6.33 (1.70)	12.64 (3.83)	9.18 (1.64)	0	2.90 (1.67)	5.23 (1.85)	5.69 (1.53)	3.65 (0.79)	2.31 (0.75)	7.96 (1.93)	2.59 (0.62)	7.04 (1.12)	3.49 (0.95)	6.86 (0.86)	2.94 (0.65)	2.39 (0.99)	1.70 (0.97)	0.91 (0.79)	0.97 (1.08)	14.41 (5.18)
YN MAT	1.30 (1.41)	4.60 (2.11)	10.83 (3.02)	8.52 (1.82)	0	5.02 (1.56)	5.38 (2.70)	4.47 (0.95)	4.40 (0.73)	2.90 (0.92)	8.17 (0.97)	2.25 (0.35)	6.83 (1.19)	2.83 (0.67)	8.03 (1.43)	4.10 (0.53)	1.47 (1.22)	1.50 (0.92)	0.90 (1.15)	2.12 (2.07)	12.68 (4.40)
BN MAT	1.48 (1.27)	5.02 (1.69)	10.35 (2.88)	8.91 (2.35)	0	3.02 (1.64)	4.13 (2.01)	5.67 (2.05)	3.33 (0.79)	2.53 (1.16)	8.64 (2.16)	2.65 (1.09)	7.08 (1.40)	4.04 (1.35)	7.12 (1.50)	3.76 (1.05)	2.48 (0.95)	1.78 (0.89)	1.03 (0.85)	1.30 (1.26)	12.01 (5.04)
	NS	P<0.01	P<0.05	NS		NS	NS	NS	P<0.05	NS	NS	NS	NS	NS	NS	P<0.01	NS	NS	NS	NS	NS

Table 6.17. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of immature cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.18. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of cuneiform cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.19. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of cupuliform cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.20. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of cuneiform plus cupuliform cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.21. Comparison of the mean percentage values (± 1 S.D.) of the subunits of the U.S. fractions of mature cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

TABLE 6.17

U.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
IMM	0.62 (0.83)	2.15 (0.74)	4.33 (1.07)	4.79 (1.73)	0	2.72 (0.96)	3.06 (0.98)	4.55 (1.03)	3.04 (0.53)	2.84 (0.81)	4.87 (0.87)	2.92 (0.30)	5.84 (1.05)	3.80 (0.68)	9.26 (1.51)	5.22 (1.05)	6.14 (1.58)	6.17 (1.37)	3.30 (0.91)	5.61 (1.99)	14.39 (3.63)
YN IMM	0.27 (0.32)	2.64 (1.04)	3.89 (0.78)	5.45 (1.31)	0	2.34 (0.95)	3.19 (1.43)	4.42 (1.21)	2.89 (0.46)	2.28 (0.71)	5.14 (0.78)	2.86 (0.53)	4.96 (0.68)	3.82 (0.84)	8.18 (0.81)	4.72 (0.89)	5.21 (0.83)	5.61 (0.89)	3.49 (0.72)	7.44 (1.11)	18.09 (4.41)
BN IMM	0.33 (0.36)	2.49 (1.50)	3.21 (1.60)	4.58 (2.17)	0	1.99 (1.19)	2.73 (0.94)	4.01 (1.50)	2.42 (0.54)	2.09 (0.59)	4.82 (1.49)	2.89 (0.77)	5.40 (0.98)	3.88 (0.69)	9.48 (1.34)	5.16 (1.07)	5.70 (1.25)	5.43 (1.64)	4.24 (1.20)	10.49 (2.33)	18.14 (5.66)
	NS	NS	P<0.05	NS		NS	NS	NS	P<0.01	P<0.05	NS	NS	NS	NS	P<0.05	NS	NS	NS	P<0.05	P<0.01	NS

TABLE 6.18

CUN	0.02 (0.04)	2.59 (0.30)	3.30 (0.32)	5.64 (1.38)	0	1.22 (0.55)	1.06 (0.50)	3.78 (1.22)	2.44 (0.34)	2.64 (0.66)	4.77 (0.84)	2.85 (1.02)	6.35 (0.51)	4.24 (0.42)	9.26 (0.78)	5.32 (0.43)	6.30 (0.51)	6.68 (0.61)	4.64 (0.72)	9.58 (1.48)	17.28 (4.11)
YN CUN	0.33 (0.41)	1.88 (0.95)	3.37 (1.08)	4.26 (1.31)	0	1.74 (1.17)	1.76 (0.89)	3.58 (1.12)	2.88 (0.47)	2.73 (0.82)	4.24 (1.03)	2.32 (0.59)	6.74 (0.79)	4.23 (0.68)	10.73 (1.49)	6.52 (1.27)	5.27 (1.03)	5.83 (1.00)	3.44 (1.22)	4.67 (1.78)	18.22 (5.23)
BN CUN	0.30 (0.47)	2.31 (1.32)	3.94 (1.36)	4.33 (1.43)	0	2.51 (0.77)	2.91 (0.99)	3.76 (1.22)	2.37 (0.68)	2.20 (0.65)	4.43 (1.18)	2.84 (0.65)	5.50 (1.28)	3.82 (1.18)	8.95 (1.35)	5.12 (1.05)	5.79 (0.95)	6.14 (0.91)	4.11 (0.93)	13.70 (3.76)	16.68 (3.17)
	NS	NS	NS	NS		P<0.01	P<0.01	NS	NS	NS	NS	NS	P<0.05	NS	P<0.01	P<0.01	NS	NS	NS	P<0.01	NS

TABLE 6.19

CUN	0.57 (0.41)	3.83 (0.98)	4.73 (1.64)	4.83 (1.51)	0	3.01 (0.90)	2.96 (1.45)	3.81 (1.71)	2.40 (1.31)	2.87 (1.23)	3.76 (1.33)	2.48 (0.80)	5.32 (0.89)	3.71 (0.85)	8.78 (0.73)	5.41 (0.93)	6.45 (1.38)	5.94 (1.03)	3.68 (1.16)	5.71 (1.62)	14.90 (6.68)
YN CUP	0.13 (0.15)	2.83 (0.71)	3.30 (1.91)	3.53 (1.18)	0	1.87 (0.93)	1.27 (0.72)	4.17 (0.64)	2.57 (0.72)	1.83 (0.40)	3.98 (1.51)	← 8.85 (0.55)	→	3.92 (0.22)	9.22 (1.48)	5.60 (0.44)	6.63 (0.40)	7.93 (0.78)	4.52 (0.74)	13.93 (1.86)	15.10 (3.71)
BN CUP	0.41 (0.57)	2.07 (1.46)	3.42 (1.35)	4.19 (1.59)	0	1.63 (1.23)	1.44 (1.28)	3.14 (1.68)	2.45 (0.75)	2.62 (1.34)	3.67 (1.66)	2.54 (1.08)	5.87 (1.59)	3.50 (0.98)	9.65 (1.83)	5.21 (0.89)	5.91 (1.02)	5.91 (1.06)	3.51 (0.76)	9.08 (3.40)	15.59 (4.82)
	NS	P<0.01	NS	NS		P<0.05	P<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	P<0.05	NS	P<0.01	NS

TABLE 6.20

CUN CUP	0.10 (0.19)	1.57 (0.30)	3.22 (0.58)	4.43 (0.87)	0	1.18 (0.68)	1.24 (0.99)	2.87 (0.95)	1.96 (0.68)	2.05 (0.97)	5.59 (1.03)	2.47 (0.64)	5.57 (0.93)	4.29 (0.35)	9.60 (0.57)	6.59 (1.10)	5.64 (1.38)	6.45 (0.46)	3.84 (0.92)	8.39 (5.33)	18.81 (6.17)
YN CUN CUP	0.48 (0.42)	2.16 (0.70)	4.21 (0.99)	4.90 (1.01)	0	1.49 (1.11)	2.86 (0.41)	3.69 (0.84)	2.48 (0.65)	1.90 (0.46)	4.92 (0.44)	2.56 (1.21)	5.44 (1.20)	3.79 (0.63)	8.47 (1.35)	5.35 (1.19)	5.59 (0.66)	5.33 (0.57)	3.06 (0.70)	5.63 (2.41)	18.11 (4.41)
BN CUN CUP	0.29 (0.36)	2.04 (0.95)	3.44 (1.28)	3.91 (1.18)	0	1.61 (0.83)	1.57 (1.33)	2.14 (0.89)	2.04 (0.66)	2.38 (0.66)	4.41 (0.99)	2.43 (0.80)	5.58 (1.08)	3.87 (0.86)	9.34 (1.05)	5.74 (1.49)	6.20 (0.89)	6.23 (0.92)	4.90 (0.97)	16.36 (4.42)	17.11 (4.76)
	NS	NS	NS	NS		NS	P<0.05	P<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	P<0.05	P<0.01	P<0.01	NS

TABLE 6.21

MAT	0.33 (0.46)	1.76 (1.30)	2.82 (1.37)	4.20 (1.69)	0	2.12 (1.46)	2.71 (1.14)	3.55 (1.48)	2.51 (0.68)	2.45 (0.80)	4.12 (1.20)	2.95 (0.72)	6.11 (0.89)	3.58 (0.77)	9.37 (1.32)	5.45 (1.42)	6.10 (1.01)	6.73 (1.40)	4.07 (0.97)	10.88 (4.65)	17.45 (5.18)
YN MAT	0.30 (0.08)	2.02 (1.34)	2.60 (1.05)	3.82 (1.35)	0	3.07 (0.57)	1.98 (1.63)	4.98 (0.87)	2.08 (1.00)	2.65 (1.10)	4.90 (0.98)	2.97 (0.93)	6.23 (1.24)	4.63 (0.57)	9.50 (0.50)	5.27 (0.50)	5.53 (0.06)	6.07 (0.51)	4.25 (0.73)	10.12 (0.90)	12.43 (1.20)
BN MAT	0.43 (0.55)	1.67 (0.96)	3.09 (1.31)	3.74 (1.41)	0	1.76 (1.09)	2.37 (1.16)	3.42 (1.25)	2.38 (0.74)	2.36 (0.84)	4.17 (0.94)	2.78 (0.73)	5.74 (1.04)	3.98 (0.81)	8.83 (1.30)	5.67 (1.13)	6.11 (0.80)	6.46 (1.28)	4.47 (0.93)	11.67 (4.07)	15.42 (4.66)
	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	P<0.05	NS	NS	NS	NS	NS	NS	P<0.05

Table 6.22. Comparison of the mean percentage values ($\pm$ 1 S.D.) of subunits of the W.S. fractions of all cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

Table 6.23. Comparison the the mean percentage values ($\pm$ 1 S.D.) of subunits of the U.S. fractions of all cortical cataracts on different nuclear backgrounds; clear, yellow and brown. Analysis of variance was carried out to determine the statistical significant difference.

TABLE 6.22

W.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
Cort.	0.86 (0.78)	5.96 (1.51)	11.49 (2.05)	9.52 (1.73)	0	3.56 (1.48)	6.26 (1.88)	5.87 (1.49)	3.57 (0.70)	2.86 (0.93)	8.06 (1.66)	2.77 (2.50)	7.70 (1.55)	3.93 (1.12)	7.66 (1.31)	3.63 (1.05)	2.47 (0.92)	1.48 (1.14)	0.70 (0.74)	0.74 (0.94)	11.46 (5.39)
YN Cort.	1.94 (1.95)	5.93 (2.14)	10.57 (3.18)	9.69 (1.56)	0	3.96 (1.18)	6.99 (1.53)	5.51 (1.23)	3.59 (0.76)	2.88 (0.64)	7.27 (1.27)	2.65 (0.67)	6.71 (1.57)	3.80 (0.98)	7.34 (1.15)	3.45 (0.92)	2.05 (1.01)	2.06 (1.57)	0.80 (0.75)	1.36 (1.04)	10.59 (3.40)
BN Cort.	1.46 (1.31)	5.67 (1.77)	10.60 (2.85)	9.82 (2.22)	0	3.52 (1.78)	5.44 (1.78)	5.56 (1.46)	3.70 (1.01)	2.67 (1.89)	7.83 (1.79)	2.62 (1.79)	7.14 (1.41)	3.96 (1.04)	7.41 (1.34)	3.56 (0.91)	2.32 (0.93)	1.68 (1.13)	0.85 (0.81)	0.96 (1.13)	11.22 (4.34)
Stat. sig P <	0.01	-	-	-		-	0.01	-	-	-	0.05	-	0.01	-	-	-	-	-	-	0.05	-

TABLE 6.23

U.S.	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
Cort.	0.38 (0.53)	2.10 (1.25)	3.40 (1.44)	4.52 (1.60)	0	2.14 (1.33)	2.51 (1.26)	3.72 (1.42)	2.52 (1.78)	2.56 (0.90)	4.38 (1.22)	2.80 (0.70)	5.87 (1.04)	3.77 (0.74)	9.02 (1.92)	5.50 (1.26)	6.07 (1.20)	6.50 (1.23)	3.87 (1.02)	8.55 (4.86)	16.83 (4.80)
YN Cort.	0.32 (0.36)	3.26 (0.87)	3.65 (1.08)	4.70 (1.36)	0	2.01 (1.09)	2.41 (1.33)	4.08 (1.13)	2.71 (0.60)	2.35 (0.78)	4.77 (0.66)	2.60 (0.72)	5.88 (1.15)	4.01 (0.73)	9.28 (1.60)	5.47 (1.25)	5.41 (0.88)	5.85 (1.04)	3.59 (0.98)	7.15 (3.04)	17.55 (4.67)
BN Cort.	0.36 (0.48)	2.07 (1.25)	3.40 (1.39)	4.10 (1.55)	0	1.90 (1.08)	2.21 (1.27)	3.34 (1.43)	2.35 (0.54)	2.35 (0.88)	4.24 (1.29)	2.72 (0.82)	5.64 (1.21)	3.82 (0.93)	9.21 (1.43)	5.41 (1.15)	5.95 (0.96)	6.11 (1.22)	4.23 (1.11)	11.95 (4.30)	16.42 (4.60)
Stat. sig P <	-	-	-	0.05		-	-	0.01	0.01	-	-	-	-	-	-	-	0.01	0.05	0.01	0.01	0.01

Little variability is shown by group II subunits although a number of trends are apparent. In the W.S. fraction, lenses with a yellow nucleus and either cuneiform or cupuliform opacities show high percentage values while in the U.S. fraction these lenses have a low percentage value for that subunit. The reverse is true for yellow lenses with immature or mature cortical involvement - a low percentage value in the W.S. and high percentage value in the U.S. fraction. In the case of the W.S. fraction of mature cataracts the difference in value is statistically significant. Subunit II_2 shows some similar tendencies in that it is yellow nuclear cataracts which, in the case of immature and cuneiform plus cupuliform in the W.S. fraction, have the lowest value, while in the case of cuneiform and cupuliform involvements, they have the highest value. In mature cortical cataract, this subunit decreases significantly with increasing nuclear colour. In the U.S. fraction, subunit II_2 decreases significantly with increasing nuclear colour on an immature cortical background in comparison to the situation in the W.S. fraction where the lowest value is observed on a yellow and the highest value on a brown nuclear background; this difference is significant. No significant difference is shown by subunit II_3 in either the W.S. or U.S. fractions but in the former it may be observed that values are consistently high in lenses with cupuliform involvement.

In group III, a number of linear increases and decreases with respect to nuclear colour are observed. On an immature cortical background, subunits III_1 and III_4 increase (not significantly) in the W.S. fraction while in the U.S. fraction those two subunits plus III_3 and III_5 decrease - III_4 and III_5 decreasing significantly - with increasing nuclear/

nuclear colour. Subunits III_2 and III_6 have low values in the W.S. fraction and high values in U.S. fraction of yellow nuclear cataracts with immature cortical involvements. In the presence of cuneiform opacities, subunits III_3 and III_6 decrease with increasing nuclear colour in the W.S. fraction although not significantly and are invariant in the U.S. fraction. In the U.S. fraction subunits III_1 and III_2 increase significantly with increasing nuclear colour while in the W.S. fraction although they show a decrease in percentage value in lenses with nuclei compared to those with no nuclear involvement, III_1 has a low, but not significantly so, value in yellow nuclear cataracts and III_2 a significantly high value in yellow nuclear cataracts. On a cupuliform background, subunit III_2 of the W.S. and III_1 of the U.S. each decrease in value with increasing nuclear colour, the latter showing statistical significance. Subunit III_6 of the W.S. is significantly low in yellow nuclear cataracts plus cupuliform opacities while in the U.S. it is invariant but consistently low compared to other cortical types. Subunit III_2 is significantly high in yellow lenses with cuneiform plus cupuliform opacities in both W.S. and U.S. fractions, as is subunit III_3 of the U.S. Subunits III_5 of the W.S. and III_6 of both the W.S. and U.S. fractions each decrease with increasing nuclear colour in lenses with cuneiform plus cupuliform opacities, the former two showing statistical significance. Subunit III_4 of the W.S. fraction is the only one showing statistically significant difference in lenses with mature cortical involvement with a high value observed in lenses with yellow nuclei; this subunit is lower in the U.S. fraction of yellow nuclear cataracts with mature cortical involvement. Also in lenses with yellow nuclei and mature/

mature cortical involvement, subunits III_1 and III_5 are high in both W.S. and U.S. while subunits III_2 and III_4 are high in the W.S. and low in the U.S.

There is little variability in group IV subunits except for IV_2 which in lenses with cuneiform opacities shows a significantly low value in lenses with a brown nucleus both in the W.S. and U.S. fractions. This subunit also shows significant difference in the W.S. fraction of lenses with immature cortical involvement, lenses with no nuclear involvement having the highest value and lenses with yellow nuclei the lowest. This arrangement of values is also the case in the U.S. fraction but no statistical significance is observed. Subunit IV_3 exhibits significant difference in the U.S. fraction of lenses with mature cortical involvement, lenses with yellow nuclei having the highest value; in the W.S. fraction this subunit is low, but not significantly so, in lenses with yellow nuclei.

Few linear trends can be observed in group V subunits except in the case of I_1 and V_2 of the W.S. fraction in the case of cuneiform plus cupuliform cataracts in which these subunits both decrease significantly with increasing nuclear colour. Also within this group of cataracts, subunits V_4 and V_6 of the W.S. show significant difference with yellow nuclear lenses exhibiting high values while V_4, V_5 and V_6 of U.S. fraction of yellow nuclear lenses exhibit significantly low values. In lenses with immature cortical involvement, subunits V_4 and V_6 of the W.S. show significant difference with high values seen in lenses with yellow nuclei and subunits V_5 and V_6 in the U.S. show a significant increase with increasing nuclear colour. Subunits V_3 and V_4 in cuneiform cataract exhibit low values both in the W.S. and/

and U.S. fractions of lenses with a yellow nucleus; the difference is significant in both cases in the W.S. fraction. In this group of cataracts, subunits V_1 and V_2 are low, but not significantly so, in the W.S. but significantly low in the U.S. fraction. Those lenses with cupuliform opacities and a yellow nucleus show significantly high values for subunits V_4 and V_6 of the U.S. other subunits of group V also showing high, but not significantly high, values in the U.S.; the total value in the W.S. fraction is higher than that of lenses with brown or no nuclear involvement. Mature cataracts show a significant difference only for subunit V_2 of the W.S. the value in lenses with yellow nuclei being high while it tends to be low in the U.S. Subunit V_1 is high in both W.S. and U.S. while subunits V_3 and V_4 are low in both. In every case both in the W.S. and U.S. fractions, except in the W.S. of cupuliform cataracts, the level of V_6 is markedly higher in brown nuclear compared to lenses with no nuclear cataract.

For each cataract type, there is a wide range of values for subunit VI as reflected in the standard deviation. Thus, although the average values are markedly different, statistically they are not significant except in the case of the W.S. fraction of cuneiform cataracts and the U.S. fraction of mature cataracts.

Tables 6.22 and 6.23, the average subunit values for all cataracts of specific nuclear types regardless of cortical involvement, do not reflect the situation seen in the smaller subgroups however. Little variation is seen in groups II and VI in both W.S. and U.S. as in the smaller subgroup, but group VI of the W.S. also shows little variation which was not the case in the subgroups. Rather more variation/

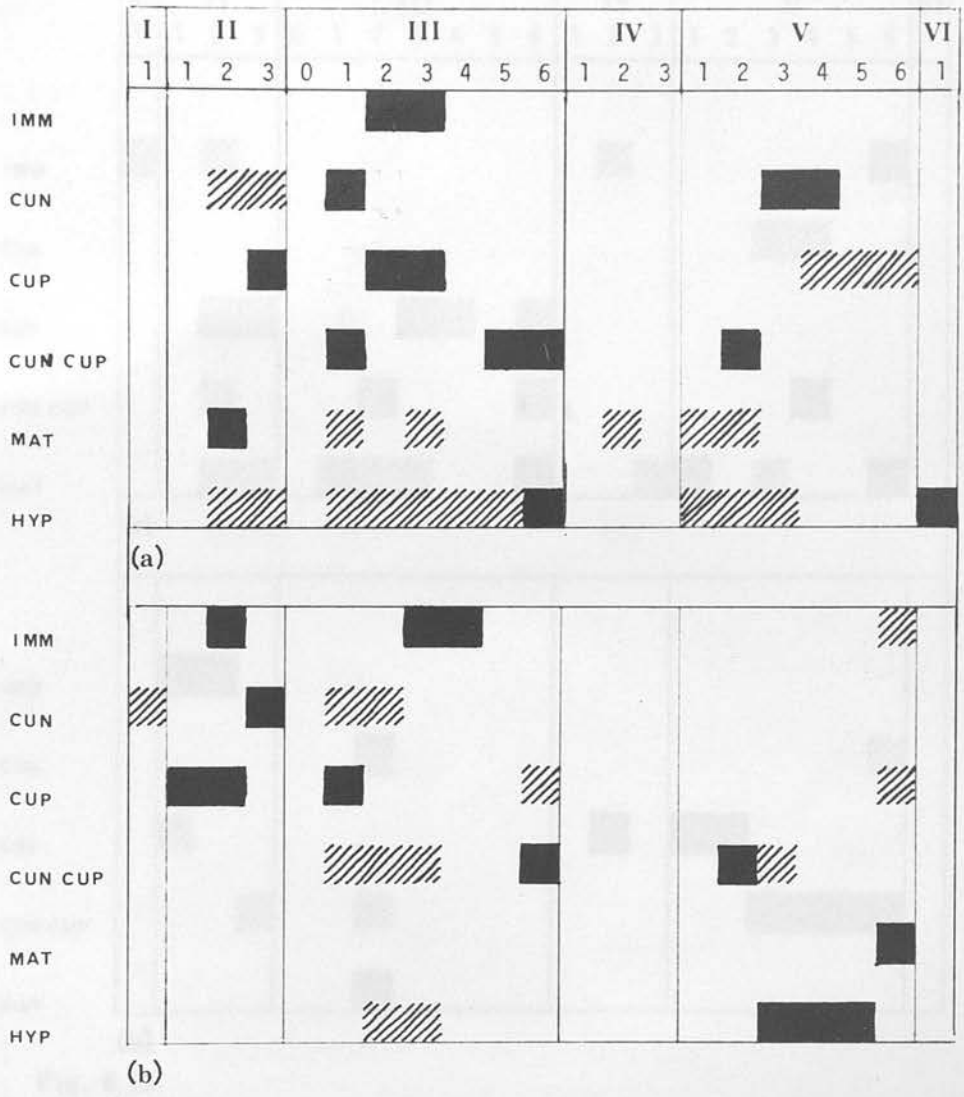


Fig. 6.11

Schematic representation of Tables 6.4 and 6.7 indicating which sub units of (a) the W.S. fraction and (b) the U.S. fraction of various categories of cortical cataract on a clear nuclear background are high (■) and low (▨) respectively.

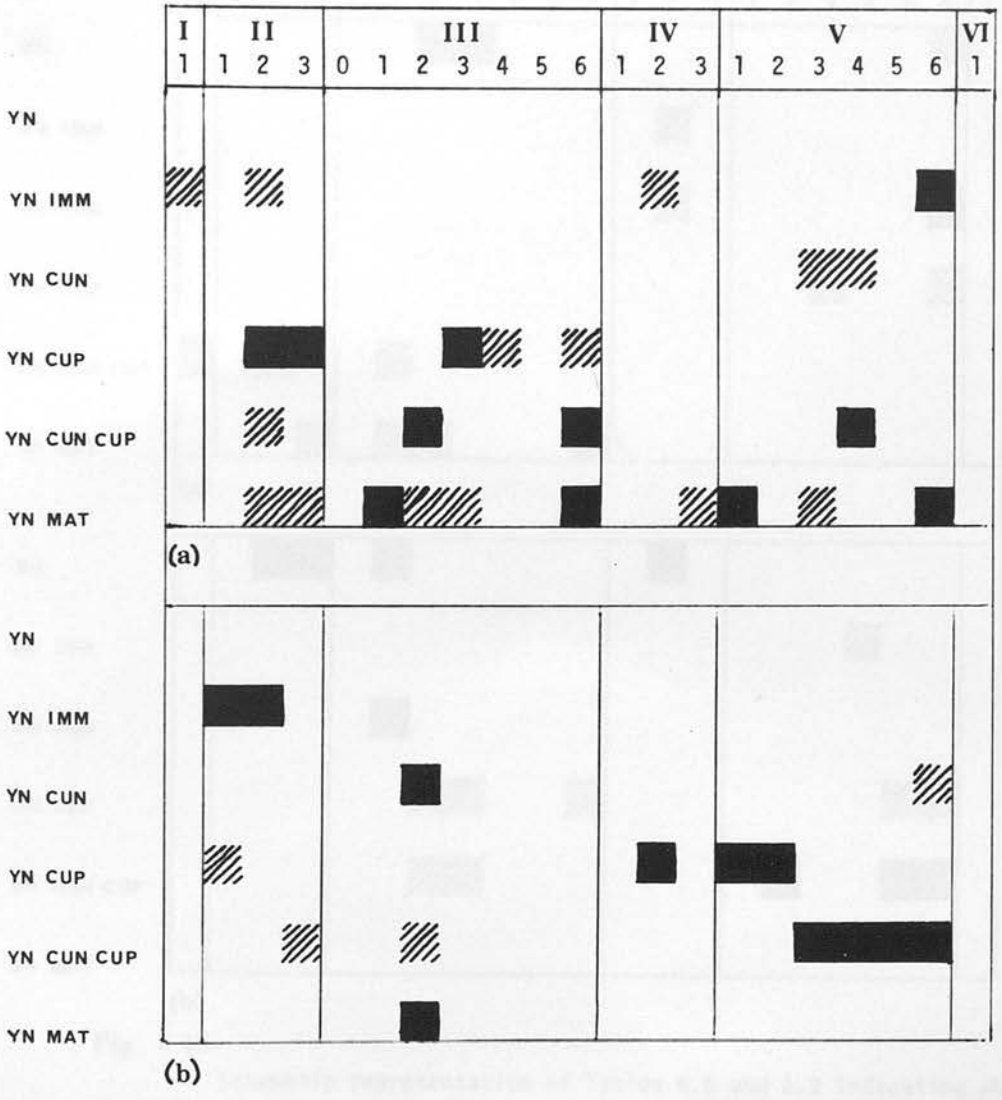


Fig. 6.12

Schematic representation of Tables 6.5 and 6.8 indicating which of (a) the W.S. fraction and (b) the U.S. fraction of various categories of cortical cataract on a yellow nuclear background are present at high (■) or low (▨) levels respectively.

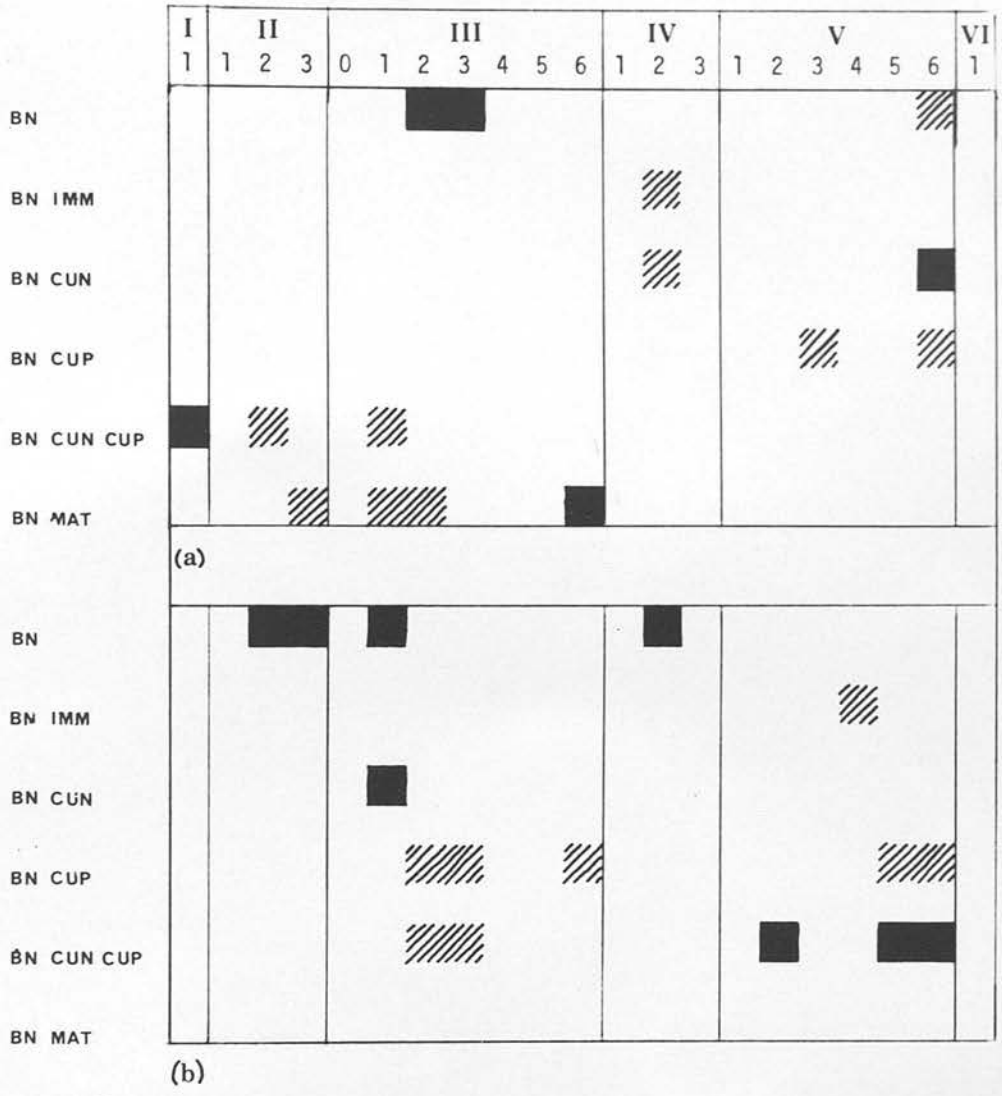


Fig. 6.13

Schematic representation of Tables 6.6 and 6.9 indicating which sub units of (a) the W.S. fraction and (b) the U.S. fraction of various categories of cortical cataract on a brown nuclear background are present at high (■) and low (▨) levels respectively.

Fig. 6.14. Schematic representation of Tables 6.12 and 6.17 indicating which subunits of (a) the W.S. fraction and (b) the U.S. fraction are raised (■) or lowered (▨) in immature (IMM) cortical cataracts differing in nuclear colour; clear, yellow (YN) or brown (BN).

Fig. 6.15. Schematic representation of Tables 6.13 and 6.18 indicating which subunits of (a) the W.S. fraction and (b) the U.S. fraction are raised (■) or lowered(▨) in cuneiform (CUN) cataracts differing in nuclear colour; clear, yellow (YN) or brown (BN).

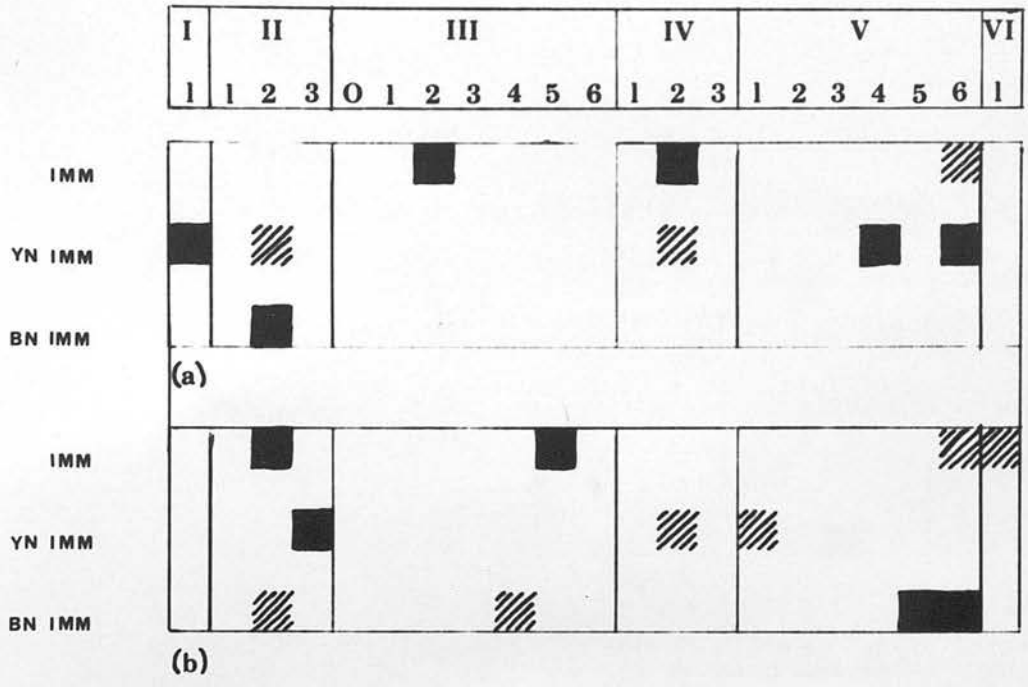


Fig. 6.14

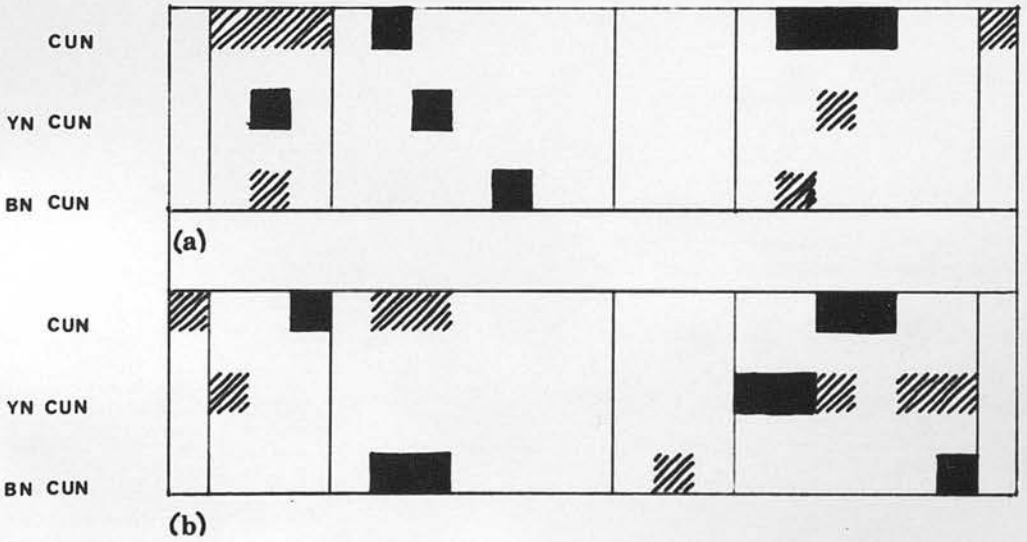


Fig. 6.15

Fig. 6.16. Schematic representation of Tables 6.14 and 6.19 indicating which subunits of (a) the W.S. fraction and (b) the U.S. fraction are raised (■) or lowered (▨) in cupuliform (CUP) cataracts differing in nuclear colour, clear, yellow (YN) or brown (BN).

Fig. 6.17. Schematic representation of Tables 6.15 and 6.20 indicating which subunits of (a) the W.S. fraction and (b) the U.S. fraction are raised (■) or lowered (▨) in cuneiform plus cupuliform (CUN CUP) cataracts differing in nuclear colour; clear, yellow (YN) or brown (BN).

Fig. 6.18. Schematic representation of Tables 6.16 and 6.21 indicating which subunits of (a) the W.S. fraction and (b) the U.S. fraction are raised (■) or lowered (▨) in mature (MAT) cortical cataracts differing in nuclear colour; clear, yellow (YN) and brown (BN).

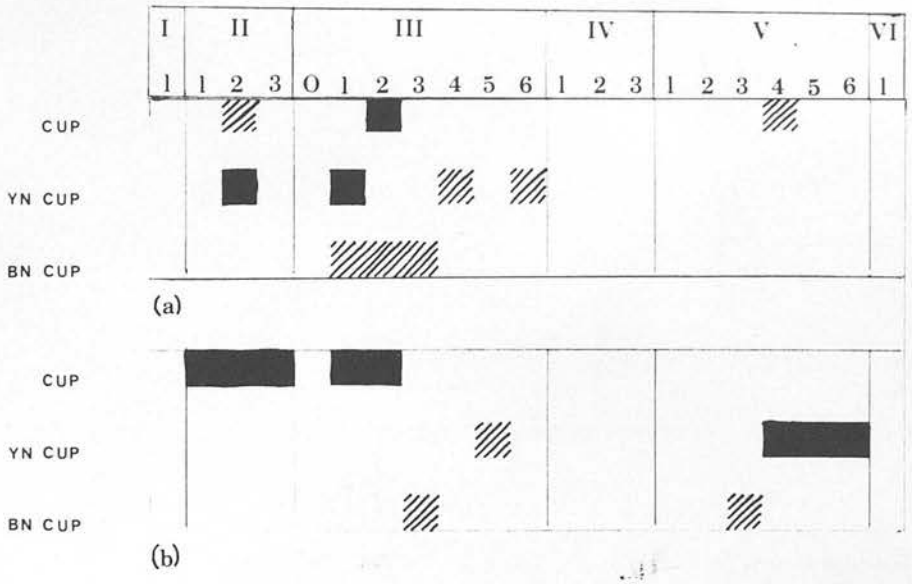


Fig. 6.16

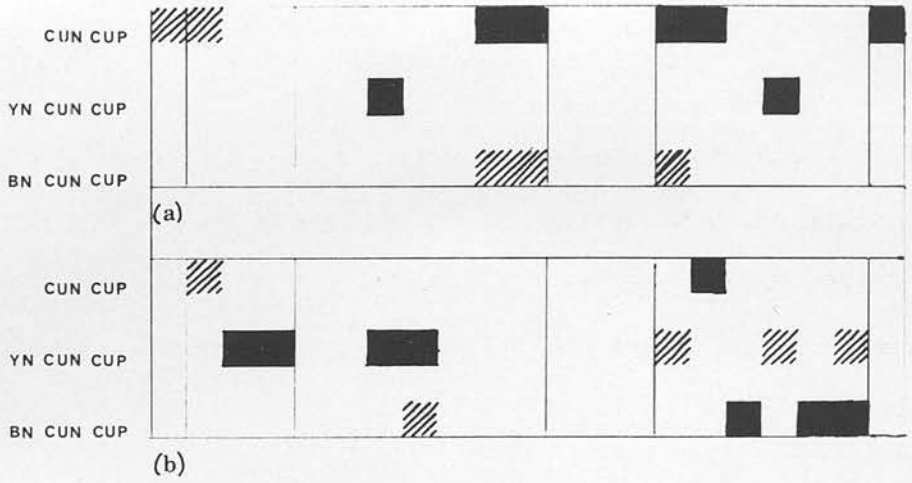


Fig. 6.17

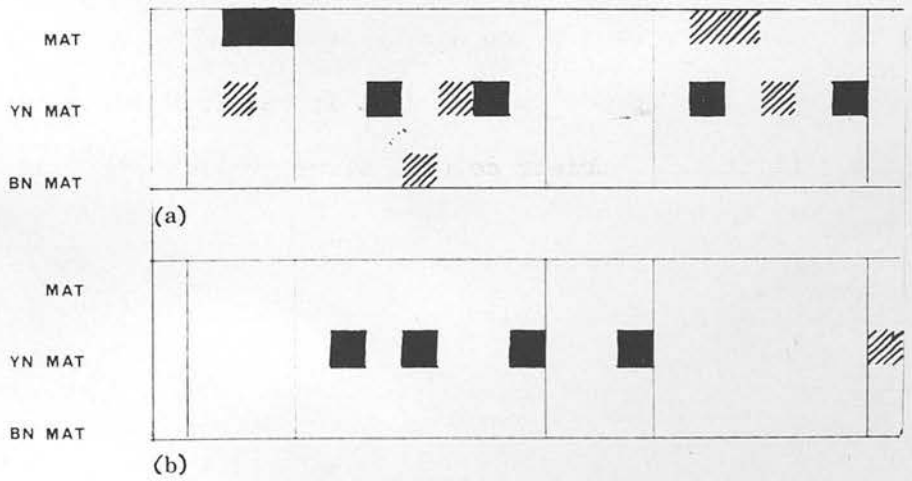


Fig. 6.18

Fig. 6.19. Schematic representation of Table 24 indicating which subunits of (a) the W.S. fraction and (b) the U.S. fraction are raised (■) or lowered (▨) in specific categories of cataracts when compared to both cataracts of similar nuclear colour but differing cortical involvement and cataracts of different nuclear colour but similar cortical involvement.

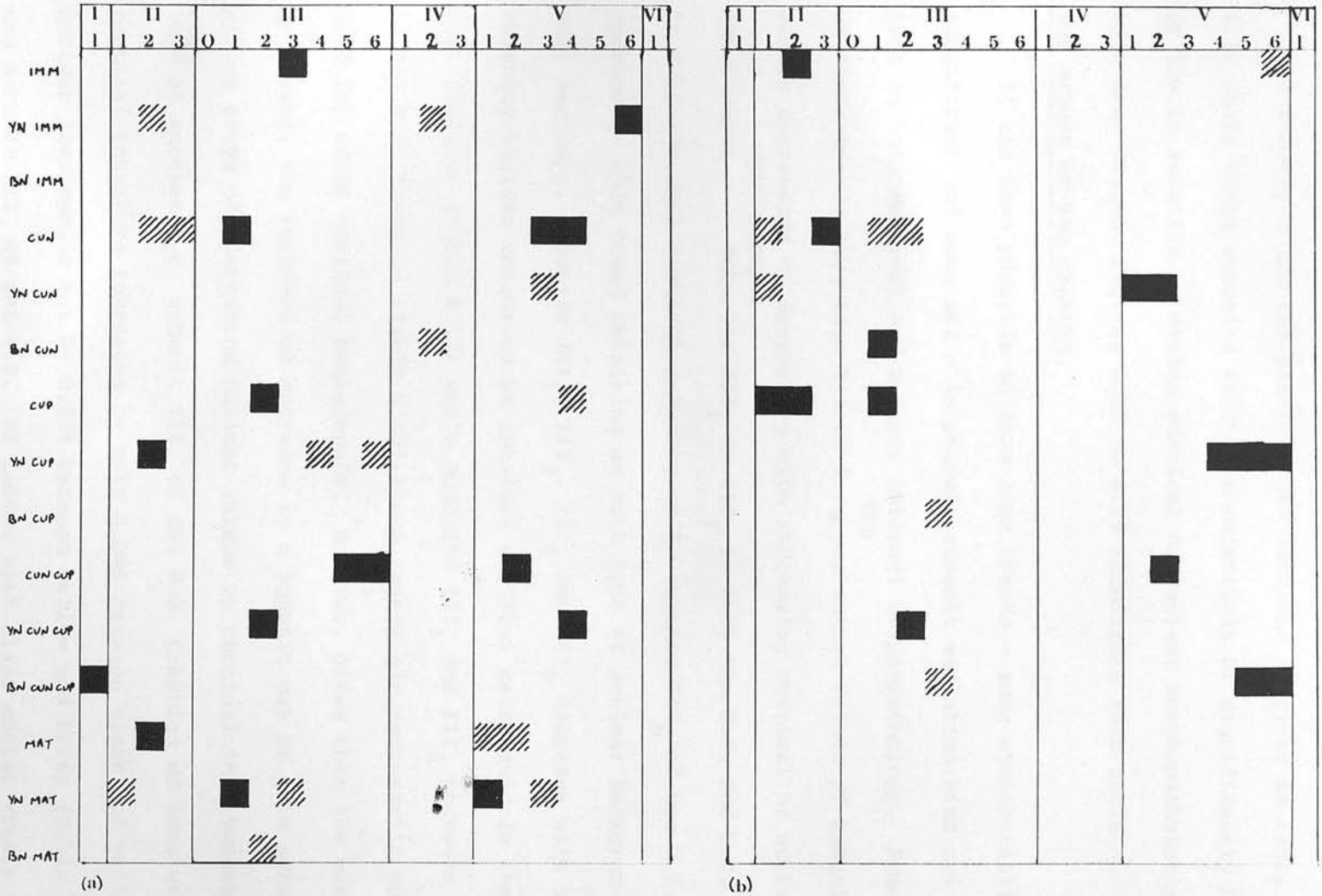

I
II
III
IV
V
VI
1
1 2 3
0 1 2 3 4 5 6
1 2 3
1 2 3 4 5 6
1
IMM
YN IMM
BN IMM
CUN
YN CUN
BN CUN
CUP
YN CUP
BN CUP
CUN CUP
YN CUN CUP
BN CUN CUP
MAT
YN MAT
BN MAT
(a)
(b)

Fig. 6.19

variation is seen in groups III and VI of the U.S.

A summary of the two previous two sections is given in table 6.24 where those subunits which are relatively or significantly high or low in relation to either cortical or nuclear morphopathologies have been listed. Figures 6.11 to 6.19 illustrate this table.

THE NATURE OF THE CHANGES.

It has been possible to show some trends - some statistically significant and some not - in protein subunit variation with respect to both cortical and nuclear cataract morphopathology. Some of these trends have been linear i.e. The percentage values of subunits either increasing or decreasing with increasing cortical or nuclear involvement. For example subunits III_1 to III_5, in both the W.S. and U.S., tend to decrease with cortical maturity while subunit III_6 of the W.S. increases, this trend obtaining on each type of nuclear background, and, secondly, subunits III_1, III_3, III_4 and III_5 decrease with increasing nuclear colour on an immature cortical background in the U.S. fraction (Table 6.17) while subunits III_1 and III_4 increase in the W.S. fraction (Table 6.12); these trends not necessarily holding good for other cortical backgrounds. However, given that the variation is linear, the increase or decrease in a subunit may be more marked at one stage of increasing nuclear colour or cortical involvement than at another e.g. subunit III_1 of the W.S. fraction of immature cortical cataracts increases by only 0.04% between clear and yellow nuclear involvement but by 0.57% between yellow and brown (Table 6.12) and subunit III_3 of the U.S. of lenses with clear nuclei (Table 6.7) is at least 1.21% lower in hypermature cortical cataracts than in any other kind /

Table 6.24. List of subunits of (a) the W.S. fraction and (b) the U.S. fraction which are present at relatively higher or lower levels when cataracts of a particular morphopathology are compared, first of all, to cataracts of similar cortical involvement but different nuclear colour and, secondly, to cataracts of similar nuclear colour but different cortical involvement. Thirdly, those subunits which are either high or low in each case are listed. This list is schematically represented in figures 6.11 to 6.19.

TABLE 6.24(a)

W.S.	Relative to similar cortical opacities but different nuclear backgrounds		Relative to similar nuclear backgrounds but different cortical opacities		Subunits which are common to both	
	High	Low	High	Low	High	Low
YN	-	-	-	-	-	-
BN	III_2 III_3	V_6	-	-	-	-
IMM	III_2 III_3	-	III_2 IV_2	V_6	III_2	-
YN IMM	V_6	I_1 II_2 IV_2	I_1 V_4 V_6	II_2 IV_2	V_6	II_2 IV_2
BN IMM	-	IV_2	II_2	-	-	-
CUN	III_1 V_3 V_4	II_2 II_3	III_1 V_2 V_3 V_4	II_1 II_2 II_3 VI_1	III_1 V_3 V_4	II_2 II_3
YN CUN	-	V_3 V_4	II_2 III_2	V_3	-	V_3
BN CUN	V_6	IV_2	III_4	II_2 V_2	-	-
CUP	II_3 III_2 III_3	V_4 V_5 V_6	III_2	II_2 V_4	III_2	V_4
YN CUP	II_2 II_3 III_3	III_4 III_6	II_2 III_1	III_4 III_6	II_2	III_4 III_6
BN CUP	-	V_5 V_6	-	III_1 III_2 III_3	-	-
CUN CUP	III_1 III_5 III_6 V_2	-	III_5 III_6 V_1 V_2 VI_1	I_1 II_1	III_5 III_6 V_2	-
YN CUN CUP	III_2 III_6 V_4	II_2	III_2 V_4	-	III_2 V_4	-
BN CUN CUP	I_1	II_2 III_1	-	III_5 III_6 V_1	-	-
MAT	II_2	III_1 III_3 IV_2 V_1 V_2	II_2 II_3	V_2 V_3	II_2	V_2
YN MAT	III_1 III_6 V_1 V_6	II_2 II_3 III_2 III_3 IV_3 V_3	III_2 III_5 V_2 V_6	II_2 III_4 V_4	V_6	II_2
BN MAT	III_6	II_3 III_1 III_2	-	III_3	-	-
HYP	III_6 VI_1	II_2 II_3 III_1 - III_5 V_1 V_2 V_3	-	-	-	-

TABLE 6.24(b)

U.S.	Relative to similar cortical opacities but different nuclear backgrounds		Relative to similar nuclear backgrounds but different cortical opacities		Subunits which are common to both	
	High	Low	High	Low	High	Low
YN	-	-	-	-	-	-
BN	II_2 II_3 III_1 IV_2	-	-	-	-	-
IMM	II_1 III_3 III_4	V_6	II_2 III_5	V_6 VI_1	-	V_6
YN IMM	II_1 II_2	-	II_3	IV_2 V_1	-	-
BN IMM	-	V_4	V_5 V_6	II_1 III_4	-	-
CUN	II_3	I_1 III_1 III_2	II_3 V_3 V_4	I_1 III_1 III_2	II_3	I_1 III_1 III_2
YN CUN	III_2	V_6	V_1 V_2	II_1 V_3 V_5 V_6	-	V_6
BN CUN	III_1	-	III_1 III_2 V_6	IV_2	III_1	-
CUP	II_1 II_2 III_1	III_6 V_6	II_1 II_2 II_3 III_1	-	II_1 II_2 III_1	-
YN CUP	IV_2 V_1 V_2	II_1	V_4 V_5 V_6	III_5	-	-
BN CUP	-	III_2 III_3 III_6 V_5 V_6	-	III_3 V_3	-	III_3
CUN CUP	III_6 V_2	III_1 III_2 III_3 V_3	V_2	II_1	V_2	-
YN CUN CUP	V_3 V_4 V_5 V_6	II_3 III_2	II_2 II_3 III_2 III_3	V_1 V_4 V_6	-	-
BN CUN CUP	V_2 V_5 V_6	III_2 III_3	V_3 V_5 V_6	III_3	V_5 V_6	III_3
MAT	V_6	-	-	-	-	-
YN MAT	III_2	-	III_1 III_3 III_6 IV_3	VI	-	-
BN MAT	-	-	-	-	-	-
HYP	V_3 V_4 V_5	II_2 II_3	-	-	-	-

kind of cortical cataracts.

In other instances, variation occurs in a non-linear fashion and either high or low values can be associated with particular lesions in the cortex or a particular stage of coloration in the nucleus e.g. subunit II_2 in the W.S. has a low value in lenses with cuneiform plus cupuliform involvement on any background (Tables 6.4, 6.5 and 6.6) - this is reflected in table 6.10 where cortical opacities are compared regardless of nuclear colour - and subunit III_2 in the W.S. has a high value in lenses with a yellow nucleus on cuneiform and cuneiform plus cupuliform backgrounds (Tables 6.13 and 6.14). Variations in the W.S. fraction need not be reflected in the U.S. fraction although it may be the case i.e. a decrease in value in the W.S. may or may not be accompanied by a concomitant increase in the U.S. and vice versa. But since there is, in the U.S., a decrease in group II and group III subunits, excluding III_4 and III_5, plus subunit IV_2 and an increase in the amounts of groups V and VI subunits when compared to the W.S. fraction, it may be that some changes in subunit values in the W.S. are reflected by changes in different subunits in the U.S.

ABSOLUTE VALUES.

Measurements of the absolute values of the subunits in terms of milligrams of protein per lens are difficult to determine since not all of the water soluble or urea soluble protein is extracted during the first extraction procedure of each respectively. This is the reason, that the pellets must be washed several times before the next stage of extraction. However, electrophoresis shows that the protein extracted at each wash has the same subunit composition in terms of percentage values. Therefore, the first supernatant in each case is truly representative of either/

TABLE 6.25 LIST OF MEAN VALUES ± I S.D. (IN MILLIGRAMS) OF THE PROTEIN SUBUNITS OF (a) THE W.S. FRACTIONS AND (b) THE U.S. FRACTIONS OF CATARACTOUS LENSES OF VARYING CORTICAL MORPHOPATHOLOGIES BUT WITH NO NUCLEAR INVOLVEMENT. THE FIGURES ARE BASED ON THE AVERAGE CONCENTRATION VALUES (TABLES 6.1 AND 6.2) AND THE MEAN PERCENTAGE VALUES OF THE SUBUNITS (TABLES 6.4 AND 6.7) OF THESE PARTICULAR CATARACT GROUPINGS.

CORTICAL INVOLVEMENT	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
Immature	0.40 (0.38)	2.86 (0.49)	5.02 (0.81)	4.84 (0.83)	- -	2.01 (0.36)	3.16 (0.89)	3.27 (0.63)	1.72 (0.36)	1.62 (0.29)	3.65 (0.66)	1.56 (0.29)	4.00 (1.00)	2.10 (0.44)	3.95 (0.58)	1.69 (0.41)	1.02 (0.33)	0.65 (0.47)	0.42 (0.42)	0.21 (0.29)	4.13 (2.00)
Cuneiform	0.32 (0.29)	2.24 (0.28)	4.06 (0.63)	3.65 (0.56)	- -	2.06 (0.36)	2.93 (0.68)	2.46 (0.24)	1.51 (0.31)	1.02 (0.16)	3.48 (0.25)	1.25 (0.46)	3.34 (0.49)	1.71 (0.30)	3.62 (0.62)	1.94 (0.60)	1.54 (0.39)	1.51 (0.55)	0.23 (0.33)	0.43 (0.52)	2.90 (1.38)
Cupuliform	0.32 (0.24)	2.37 (0.55)	4.02 (1.00)	4.31 (0.78)	- -	1.48 (0.39)	2.93 (0.49)	2.51 (0.50)	1.37 (0.23)	1.27 (0.26)	3.29 (0.54)	1.09 (0.35)	3.18 (0.56)	1.72 (0.51)	3.01 (0.69)	1.45 (0.40)	0.89 (0.26)	0.10 (0.13)	0.05 (0.05)	0.05 (0.06)	3.19 (1.51)
Cuneiform + cupuliform	0.29 (0.23)	2.09 (0.61)	4.02 (0.57)	3.91 (0.37)	- -	1.87 (0.49)	2.65 (0.41)	2.02 (0.75)	1.51 (0.23)	1.78 (0.38)	3.90 (0.42)	1.05 (0.28)	3.37 (0.78)	1.93 (0.56)	3.50 (0.47)	1.98 (0.33)	1.21 (0.34)	0.54 (0.48)	0.22 (0.21)	0.32 (0.35)	5.19 (1.46)
Mature	0.22 (0.10)	1.49 (0.40)	2.97 (0.90)	2.16 (0.39)	- -	0.68 (0.39)	1.23 (0.44)	1.34 (1.53)	0.86 (0.19)	0.54 (0.18)	1.88 (0.45)	0.61 (0.15)	1.66 (0.26)	0.82 (0.22)	1.62 (0.21)	0.69 (0.15)	0.56 (0.23)	0.50 (0.23)	0.21 (0.19)	0.28 (0.25)	3.39 (1.22)
Hypermature	0.13 (0.11)	0.79 (0.12)	2.13 (0.63)	1.33 (0.15)	- -	0.30 (0.25)	0.33 (0.09)	0.65 (0.31)	0.47 (0.11)	0.27 (0.10)	1.70 (0.35)	- -	1.91 (0.38)	- -	1.09 (0.28)	0.32 -	0.18 -	0.24 (0.21)	0.14 (0.13)	0.25 (0.23)	4.69 (1.36)
Immature	0.16 (0.22)	0.57 (0.20)	1.14 (0.28)	1.26 (0.46)	- -	0.72 (0.25)	0.81 (0.26)	1.20 (0.27)	0.80 (0.14)	0.75 (0.21)	1.28 (0.23)	0.77 (0.08)	1.54 (0.28)	1.00 (0.18)	2.44 (0.40)	1.38 (0.28)	1.62 (0.42)	1.63 (0.36)	0.87 (0.24)	1.48 (0.52)	3.79 (0.96
Cuneiform	0.00 (0.00)	0.45 (0.05)	0.58 (0.06)	0.99 (0.24)	- -	0.21 (0.10)	0.19 (0.09)	0.66 (0.21)	0.43 (0.06)	0.46 (0.12)	0.83 (0.15)	0.50 (0.18)	1.11 (0.09)	0.74 (0.07)	1.62 (0.14)	0.93 (0.08)	1.10 (0.09)	1.17 (0.11)	0.81 (0.13)	1.68 (0.26)	3.02 (0.72)
Cupuliform	0.14 (.10)	0.95 (0.24)	1.18 (0.41)	1.20 (0.38)	- -	0.75 (0.22)	0.74 (0.36)	0.95 (0.43)	0.60 (0.33)	0.71 (0.31)	0.93 (0.33)	0.62 (0.20)	1.32 (0.22)	0.92 (0.21)	2.18 (0.18)	1.34 (0.23)	1.60 (0.34)	1.48 (0.26)	0.91 (0.29)	1.42 (0.40)	3.70 (1.66)
Cuneiform + cupuliform	0.02 (0.04)	0.35 (0.07)	0.73 (0.13)	1.00 (0.20)	- -	0.27 (0.15)	0.28 (0.22)	0.65 (0.21)	0.44 (0.15)	0.46 (0.22)	1.26 (0.23)	0.56 (0.14)	1.26 (0.21)	0.97 (0.08)	2.17 (0.13)	1.49 (0.25)	1.27 (0.31)	1.46 (0.10)	0.87 (0.21)	1.89 (1.20)	4.24 (1.39)
Mature	0.06 (0.09)	0.34 (0.25)	0.55 (0.27)	0.82 (0.33)	- -	0.42 (0.29)	0.53 (0.22)	0.70 (0.29)	0.49 (0.13)	0.48 (0.16)	0.81 (0.24)	0.58 (0.14)	1.20 (0.17)	0.70 (0.15)	1.84 (0.26)	1.07 (0.28)	1.18 (0.20)	1.32 (0.27)	0.80 (0.19)	2.13 (0.90)	3.42 (1.02)
Hypermature	0.07 (0.06)	0.12 (0.08)	0.54 (0.32)	0.33 (0.10)	- -	0.29 (0.10)	0.22 (0.18)	0.35 (0.05)	0.42 (0.16)	0.45 (0.27)	0.93 (0.28)	- 1.88 - (0.52)		0.77 (0.23)	2.04 (0.37)	1.34 (0.37)	1.41 (0.36)	1.74 (0.58)	1.19 (0.33)	2.19 (1.01)	4.17 (1.91)

either the W.S. or U.S. fraction. Secondly, although the lens is homogenised in a known amount of buffer, each supernatant almost certainly differs in volume. Accepting these drawbacks, an attempt has been made using the average concentration values (Tables 6.1 and 6.2) and the percentage values for the W.S. and U.S. fractions of cortical cataracts (Tables 6.4 and 6.7) to construct a sample table illustrating how the absolute levels may vary in comparison to the percentage values (Table 6.26).

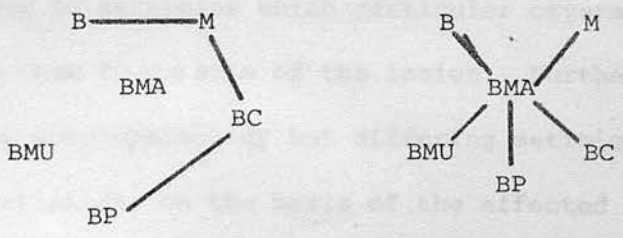
Some of the variations are similar; decreasing percentage values with decreasing concentrations give absolute values which are also decreasing; subunits III_1 to III_4 of the W.S. fraction and to a certain extent in the U.S. fraction are examples of this. However, increasing percentage values need not mean increasing absolute values, for example subunit III_6 of the W.S. while increasing proportionately remains level in absolute terms except in the case of mature and hypermature cataracts where it drops. But these are only indications of what may happen to the subunits in absolute terms based on figures achieved by combining two sets of averages. Therefore, statistically, conclusions are difficult to draw but in the future, it may prove useful to use both sets of values; proportional and absolute.

COMPARISON OF TOTAL PROFILES.

Having determined the kind of variation in particular subunits, that difference must be considered as part of the overall profile. It may be that an increase in one subunit is observed if some or all of the other subunits undergo some decrease so that, while the absolute amount of the first subunit may remain unchanged, its percentage value will increase. The converse of this may also be true. Secondly, it may/

may be that an increase in one subunit may be accompanied by a decrease in one other subunit and this might suggest some association between these two events.

By analysis of these changes and variations and identification of their association with particular cortical lesions or stages in nuclear coloration, it is hoped that each type of cataract can be shown to be identifiable by its protein profile. and in particular by those subunits which show variation (see Table 6.24). Yim (1978) used some of the data listed in this thesis and analysed them statistically using stepwise discriminant analysis; this involved computing all the subunits of either the W.S. or U.S. fractions as one resultant rather than individually. Although the set of data was less full than in this thesis, the results do support some of the findings here. Yim showed significant differences ($p < 0.05$) between certain groups of cataract based on either the W.S. or U.S. fractions. On the basis of the W.S. fraction, three pairs of cataract type were shown to be different; brown nuclear and mature cortical, mature cortical and brown nuclear plus cuneiform and, thirdly, brown nuclear plus cuneiform and brown nuclear plus cupuliform (Figure 6.20a). On the basis of the U.S. fraction, brown nuclear plus mature cortical cataracts were shown to be different from mature cortical cataracts and other cataract types involving a brown nucleus (Figure 6.20b).



a) b)

Figure 6.20. Comparison of a) the W.S. and b) the U.S. fractions of six cataract types. A line linking two groups indicates difference at a significance level of $P < 0.05$.

B = brown nuclear, M = mature cortical, BMA brown nuclear plus mature cortical, BMU = brown nuclear plus multiple cortical opacities, BP = brown nuclear plus cupuliform, BC = brown nuclear plus cuneiform (From Yim (1978)).

She also showed that yellow nuclear and brown nuclear cataracts in the presence of cuneiform or multiple cortical involvement were significantly different (Figures 6.21 and 6.22).

DISCUSSION.

It has been possible to show by comparison of the proportional values of the protein subunits in cataract that progression and maturation of the opacity is reflected by a quantitatively changing subunit composition. In the case of cortical cataracts it is sometimes possible to discern linear increases or decreases with increasing maturity although in some cases an increase or decrease may be more significant at either an earlier or later stage. Secondly, some variation from linearity may be apparently related to the site of the lesion, i.e. whether the anterior or posterior cortex is involved. In these cases, with the identification of the subunits, it may be possible to determine which particular crystallins are affected and relate them to the site of the lesion. Further, in cataracts of similar morphopathology but differing aetiology it should be possible to discriminate on the basis of the affected subunits.

With regard to nuclear cataract, it has also been the case that some/

Fig. 6.21. Computer generated result when the total protein profiles of yellow nuclear cataracts with multiple cortical involvement are compared with those of brown nuclear cataracts with multiple cortical involvement.(From Yim, 1978).

Fig. 6.22. Computer generated result when the total protein profiles of yellow nuclear cataracts with cuneiform cataract are compared with those of brown nuclear cataracts with cuneiform cataract.(From Yim, 1978).

Plot 2B

```
                                                B
 B                  BBB BB BB B    BB B  B            B   Y Y    B         Y          YYY       Y
.+....+....+....+....+....+....+2...+....+....+....+...£+....+....+....+....+.1..+....+....+....+....+...
       -2.45      -1.75      -1.05       -.350       .350       1.05       1.75       2.45       3.15       3.85
   -2.80      -2.10      -1.40      -.700       0.00       .700       1.40       2.10       2.80       3.50
```

ON THE AXIS £ INDICATES DIVIDING POINT AND NUMBERS THE GROUP MEANS

Y : YMU 1 : GROUP MEAN OF YMU

B : BMU 2 : GROUP MEAN OF BMU

Fig. 6.21

Plot 3B

```
                    B
 BB  B        B   B    BB B BB  BB   B       Y     Y   Y    Y   Y                        Y          Y
.+....+....+....+....2....+....+....+....+..£.+....+....+....+....1....+....+....+....+....+....+....+...
       -1.75      -1.05      -.350       .350       1.05       1.75       2.45       3.15       3.85       4.55
   -2.10      -1.40      -.700       0.00       .700       1.40       2.10       2.80       3.50       4.20
```

ON THE AXIS £ INDICATES DIVIDING POINT AND NUMBERS THE GROUP MEANS

Y : YC 1 : GROUP MEAN OF YC

B : BC 2 : GROUP MEAN OF BC

Fig. 6.22

some linear increases and decreases have been observed but, also, some non-linear changes are apparent and this, along with the studies of Yim (1978) suggest that coloration is not a gradual process of darkening of the nucleus (as discussed in the relevant section in the introduction) but rather a stepwise process in terms of the effects on crystallins (see also Bartholomew et al. (1980) where this is shown).

Overall, it has been shown that the different morphopathological types of cataract can be distinguished in terms of raised or lowered proportions of certain subunits, no two cataract types having simialr complements of affected subunits.

CHAPTER SEVEN

CATARACTS OF KNOWN AETIOLOGY COMPARED WITH OTHERS OF SIMILAR MORPHOPATHOLOGY

1. Cataracts from Diabetic Patients

In this study, forty seven of the 506 lenses were extracted from diabetic patients. This figure (9.29%) is in agreement with the figures cited by Caird (1973) seen in table 7.1 and is, as Caird, Pirie and Ramsell (1969) observe, in excess of the 1-2% of the population who are diabetic thus supporting the observation of Caird (1973) that cataract extractions are more frequent in the diabetic population even if the disease itself is not.

Table 7.1

Diabetes and Cataract Extraction (from Caird 1973)

Authors	No. of Extractions	% of Diabetics
Marshall (1897)	1571	2
Clegg (1920)	1660	5
Anthenisen (1936)	1717	8
Owens and Hughes (1947)	2087	11
Townes and Casey (1955)	1844	7
Caird et al. (1964)	1024	11
Norn (1967)	1714	10
Ramsell (1970)	808	9
Marquardt and Kirschbaum (1971)	361	14
Muller and Weber (1971)	1209	17
Schaffl (1971)	3976	12
	17919	9.64
This study	506	9.29

No information was available regarding the type of diabetes or the age of onset. All of the diabetic patients were over the age of fifty except one who was 23 years of age. However, the extraction of the 23 year old cataract was carried out using the technique of aspiration. Therefore, no data is presented here for that lens since they would not be comparable with those from whole lenses. It may be that these lenses from older diabetics should not be considered as true diabetic cataracts, if the hypotheses of Gray (1933), O'Brien et al (1934) and Bellows (1944) are accepted. They consider that only cataracts of diabetics less than 40 years of age are true diabetic cataracts. However, it is more likely that Romer (1912) and Anthonisen (1936) are correct in considering that diabetic cataract can be diagnosed at any age rather than exclusively under the age of forty (see chapter two section 7(i)a). It is probably the case that juvenile diabetes has a strong genetic component whereas late onset diabetes, while having a genetic basis, involves a stronger environmental interaction. The mechanisms of cataractogenesis, therefore, may or may not be the same in each case but both could be considered as diabetic cataracts. However, these lenses may not form a strictly homogeneous group, as the morphology of the cataracts in this group is varied.

Because of the small number and the varied morphology of the diabetic lenses, they must either be grouped according to nuclear colour irrespective of cortical involvement or vice versa in order to have numbers that can be tested statistically. However, neither grouping shows nay preferred morphopathology within this population of cataracts (Tables 7.2 and 7.3) although

Table 7.2.

Incidence of nuclear cataract in diabetic compared with non-diabetic patients excluding one lens with a white and one with a dark brown nucleus.

Nuclear Colour	Diabetic	Non-diabetic	
None	13	128	142
Yellow	7	74	81
Brown	25	212	237
	45	415	460

Table 7.3

Incidence of cortical cataract in diabetic compared to non-diabetic patients excluding lenses with multiple or punctate cortical opacities.

Cortical Involvement	Diabetic	Non-diabetic	
Immature	7	64	71
Cuneiform	8	58	66
Cupuliform	2	46	48
Cuneiform + Cupuliform	4	47	51
Mature	14	98	112
	35	313	348

$\chi^2 = 3.309$ Not Stat. Sig.

there is a suggestion from table 7.4 that perhaps with larger numbers some excess of cuneiform opacities would be observed. In constructing table 7.4 it has been assumed that immature and mature cortical cataracts involve, respectively, early and late symptoms of cuneiform cataracts.

Table 7.4

Incidence of cuneiform involvement in diabetic and non-diabetic patients.

Anterior Cortical Involvement	Diabetic	Non-diabetic	
Cuneiform	41	316	357
Non Cuneiform	3	41	44
None	3	102	105
	47	459	506

$\chi^2 = 7.83$ $p < 0.01$

It might be expected that nuclear colour is independent of the presence or absence of diabetes but if it is the case that anterior cortical cataracts, and in particular cuneiform cataracts, would result after diabetic insult, i.e. osmotic imbalance (Kinoshita, 1974 and Pirie, 1976) and that cupuliform (posterior subcapsular) cataract may result in the event of diabetic retinopathy (Creighton et al. 1978) then there is a case for grouping these lenses together as "diabetic" cataract if they prove to be different from non-diabetic cataracts of similar morphopathology.

Wet Weight, Dry Weight and % Dry Weight.

When the population is divided into those from diabetic and those from non-diabetic patients there is no significant difference in any of these three factors (Table 7.5).

Table 7.5

Comparison of wet weight, dry weight and % dry weight of diabetic and non-diabetic lenses.

	Wet Wt.(mg)	Dry Wt.(mg)	% Dry Wt.
Diabetic	181 $\pm$ 42	57 $\pm$ 22	31.8 11.0
Non-diabetic	188 $\pm$ 43	58 $\pm$ 21	30.8 10.1
Statistical Significance	None	None	None

However, it is also possible to make some comparisons between smaller groups both within the diabetic population and between the diabetic and non-diabetic populations. Comparing diabetic lenses, there is no significant difference in wet weight between groups of lenses either when they are grouped according to nuclear colour or when they are grouped according to cortical/

cortical involvement. There are however significant differences of both dry weight ($p < 0.01$) and percentage dry weight ($p=0.01$) when different cortical groupings are compared, but there are no significant differences between nuclear colour groupings (Figures 7.1 and 7.2).

Significant differences in wet and dry weights and percentage dry weights can be shown between diabetic and non-diabetic lenses of particular morphologies. In the nuclear groups, only those with no nuclear colour show a significant difference between diabetics and non-diabetics, the diabetics having a lower wet weight ($p < 0.05$) although diabetic yellow nuclear cataracts do show a marked decrease in wet weight (Figure 7.2). Within the cortical groupings, a marked but not significant decrease in wet weight can be seen in immature cortical and cuneiform plus cupuliform diabetic cataracts and an increase in the wet weight of cuneiform cataract. (Figure 7.1) The dry weights of cupuliform and cuneiform plus cupuliform cataractous lenses are significantly higher ($p < 0.05$ and $p < 0.01$ respectively).

It can be seen from figures 7.1 and 7.2 that the wet, dry and % dry weights of diabetic lenses follow a pattern similar to that of non-diabetic lenses, but with a greater difference between the values in both nuclear and cortical groupings. The high dry weight of lenses with cupuliform involvement is inconsistent with the microscopic studies on diabetic posterior subcapsular cataract by Streeten and Eslaghian (1978) who showed the presence of bladder cells and areas of liquefaction but the significant difference in dry weight from non-diabetic/

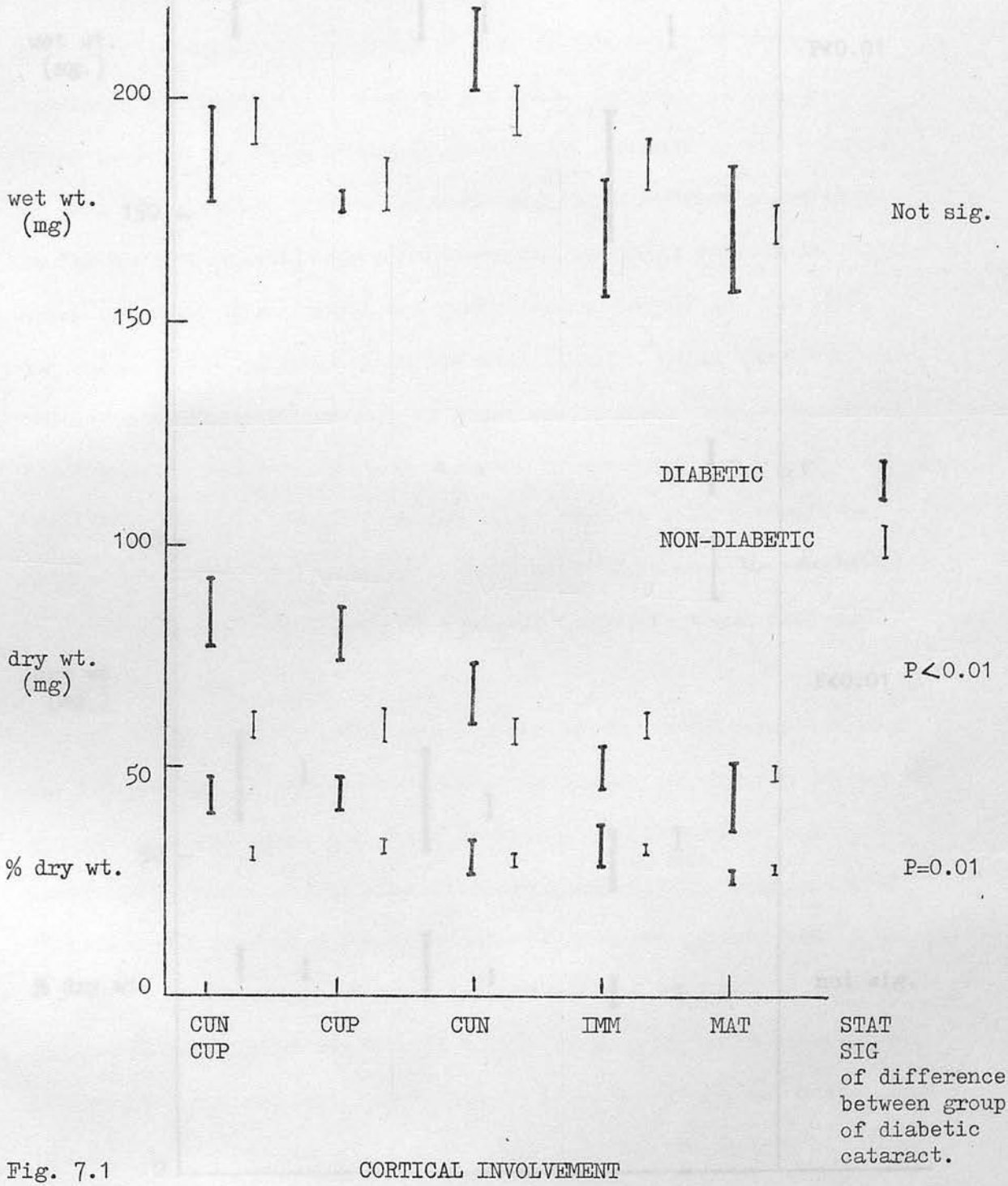


Fig. 7.1 CORTICAL INVOLVEMENT

Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) of diabetic lenses grouped accordingly to their cortical morphopathology regardless of nuclear colour. Comparisons are also made between the wet, dry and percentage dry weights of diabetic

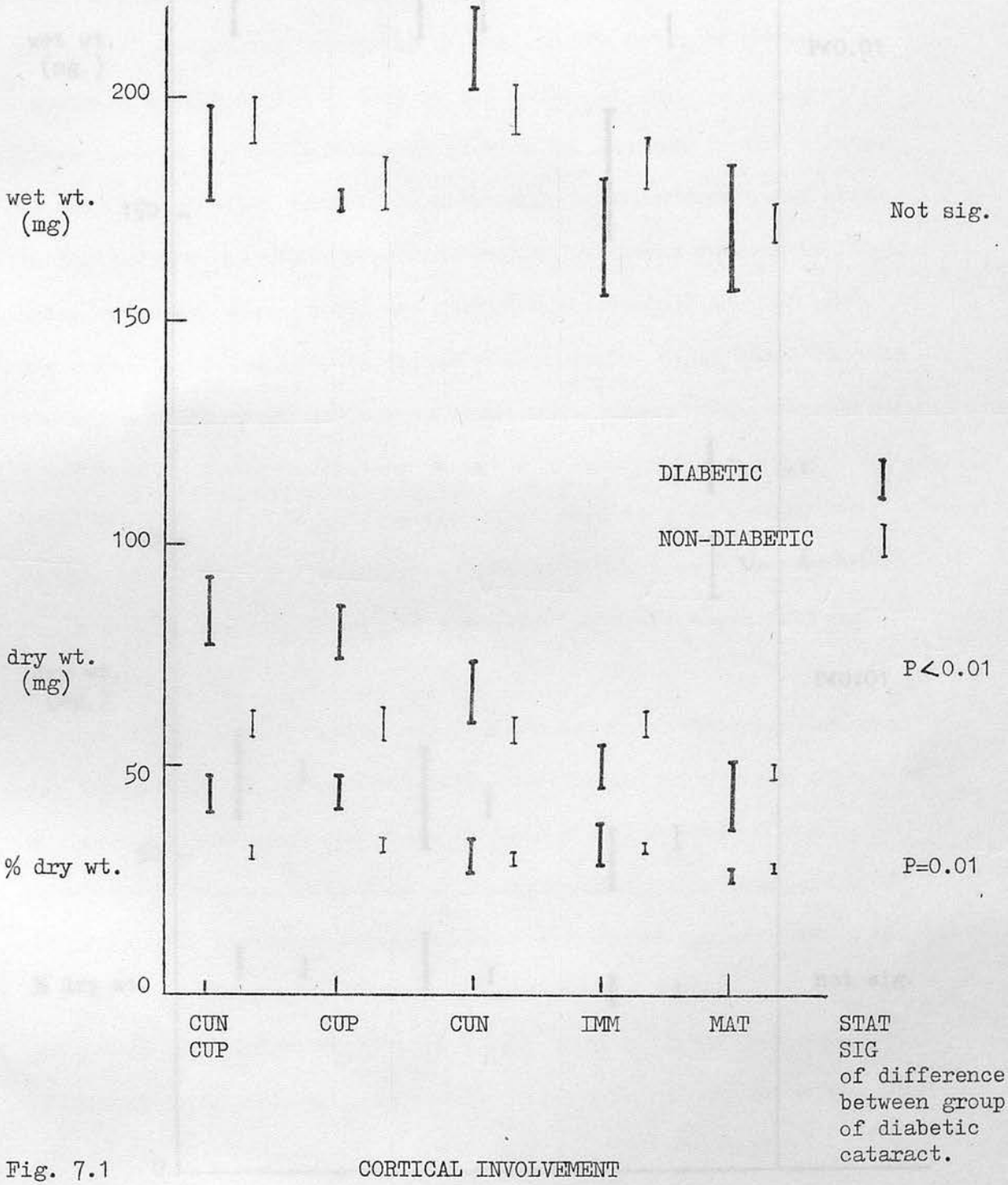


Fig. 7.1 CORTICAL INVOLVEMENT

Comparison of the wet weights, dry weights and percentage dry weights (presented as the mean $\pm$ 1 S.E.) of diabetic lenses grouped accordingly to their cortical morphopathology regardless of nuclear colour. Comparisons are also made between the wet, dry and percentage dry weights of diabetic and non-diabetic lenses of similar morphopathology.

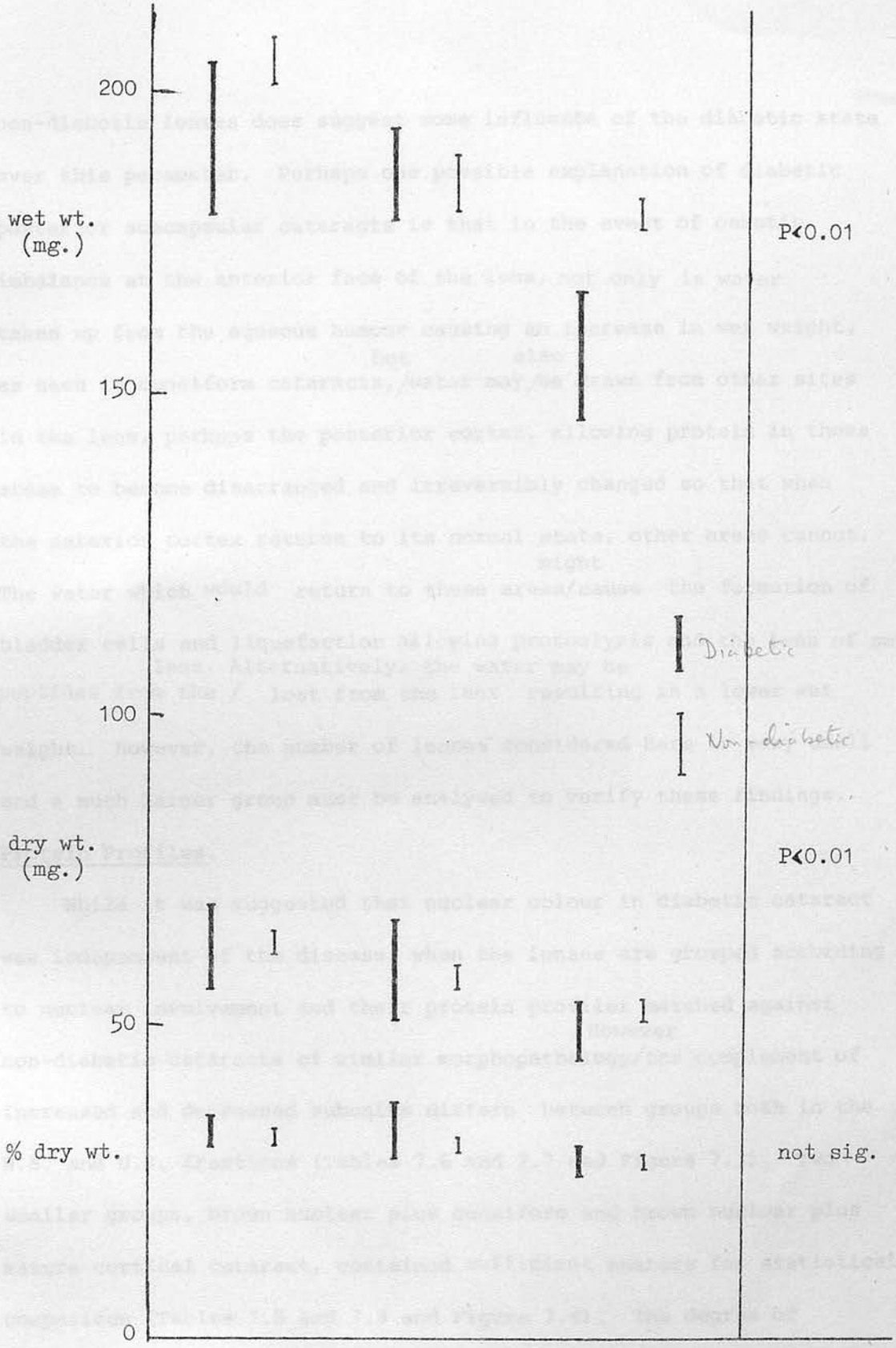


Fig. 7.2 NUCLEAR COLOUR

Comparison of the wet, dry and percentage dry weights (presented as the mean $\pm$ 1 S.D.) of diabetic lenses grouped accordingly to their nuclear colour regardless of cortical involvement. Comparisons are also made between the wet, dry and percentage dry weights of diabetic and non-diabetic lenses of similar morphopathology.

non-diabetic lenses does suggest some influence of the diabetic state over this parameter. Perhaps one possible explanation of diabetic posterior subcapsular cataracts is that in the event of osmotic imbalance at the anterior face of the lens, not only is water taken up from the aqueous humour causing an increase in wet weight, as seen in cuneiform cataracts, but also water may be drawn from other sites in the lens, perhaps the posterior cortex, allowing protein in those areas to become disarranged and irreversibly changed so that when the anterior cortex returns to its normal state, other areas cannot. The water which might return to these areas cause the formation of bladder cells and liquefaction allowing proteolysis and the loss of small peptides from the lens. Alternatively, the water may be lost from the lens resulting in a lower wet weight. However, the number of lenses considered here is very small and a much larger group must be analysed to verify these findings.

Protein Profiles.

While it was suggested that nuclear colour in diabetic cataract was independent of the disease, when the lenses are grouped according to nuclear involvement and their protein profiles matched against non-diabetic cataracts of similar morphopathology. However the complement of increased and decreased subunits differs between groups both in the W.S. and U.S. fractions (Tables 7.6 and 7.7 and Figure 7.3). Two smaller groups, brown nuclear plus cuneiform and brown nuclear plus mature cortical cataract, contained sufficient numbers for statistical comparison (Tables 7.8 and 7.9 and Figure 7.4). The degree of variation in each of these groups was much greater, in that more subunits were involved, than in the larger brown nuclear cataract group./

Table 7.6. Comparison of the mean percentage values ($\pm$ 1 S.D.) of (a) the W.S. fractions and (b) the U.S. fractions of cortical cataracts with no nuclear involvement from diabetics and non-diabetics.

Table 7.7. Comparison of the mean percentage values ($\pm$ 1 S.D.) of (a) the W.S. fractions and (b) the U.S. fractions of yellow nuclear cataracts with any cortical involvement from diabetics and non-diabetics.

TABLE 7.6

	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
Diabetic	1.07	4.31	8.60	11.32	-	3.50	7.12	6.11	3.26	3.20	7.24	3.29	8.04	4.44	8.23	4.10	2.22	1.83	0.80	1.74	8.36
	(1.06)	(1.50)	(1.42)	(2.47)	-	(1.44)	(1.10)	(0.92)	(1.24)	(0.99)	(1.91)	(0.96)	(2.97)	(0.94)	(1.53)	(1.56)	(1.61)	(1.88)	(0.65)	(1.74)	(4.26)
Nondiabetic	0.88	6.04	11.74	9.46	-	3.54	6.17	5.84	3.67	2.82	8.10	2.71	7.70	3.86	7.61	3.53	2.45	1.40	0.69	0.73	11.96
	(0.80)	(1.53)	(1.90)	(1.63)	-	(1.53)	(1.93)	(1.53)	(0.68)	(0.91)	(1.64)	(2.63)	(1.55)	(1.13)	(1.29)	(0.98)	(0.88)	(1.03)	(0.74)	(0.93)	(5.36)
Stat sig. P <	-	0.0025	0.0005	0.0025	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.05
Diabetic	0.21	1.56	3.16	4.58	-	1.61	2.54	4.14	2.29	2.72	4.61	2.76	5.66	3.86	10.82	6.33	7.12	-	9.42	-	10.42	14.64
	(0.29)	(0.82)	(1.25)	(1.83)	-	(1.45)	(1.80)	(1.18)	(1.32)	(1.18)	(1.29)	(1.35)	(1.13)	(1.32)	(2.85)	(1.51)	(1.93)		(1.28)		(3.02)	(3.80)
Nondiabetic	0.38	2.12	3.43	4.51	-	2.13	2.50	3.64	2.57	2.55	4.39	2.77	5.92	3.80	8.96	5.50	6.05	6.52	3.89	8.63	17.00
	(0.55)	(1.28)	(1.42)	(1.58)	-	(1.34)	(1.25)	(1.43)	(0.77)	(0.90)	(1.23)	(0.57)	(1.03)	(1.00)	(1.97)	(1.30)	(1.17)	(1.24)	(1.01)	(4.96)	(4.86)
Stat sig. P <	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	0.01	-	-	-	-

TABLE 7.7

	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
Diabetic	1.48	4.50	12.82	9.58	-	4.24	5.82	4.64	3.78	3.02	7.20	2.64	8.20	3.52	7.57	4.13	2.97	2.10	1.00	0.58	10.12
	(1.10)	(2.40)	(3.60)	(1.09)	-	(1.60)	(2.62)	(0.83)	(0.95)	(0.88)	(2.09)	(0.77)	(0.61)	(0.90)	(2.15)	(1.86)	(0.47)	(0.96)	(1.01)	(0.77)	(1.43)
Nondiabetic	2.00	5.99	10.39	9.70	-	3.98	6.98	5.61	3.57	2.89	7.23	2.65	6.57	3.83	7.37	3.24	1.96	2.06	0.78	1.44	10 63
	(2.04)	(2.16)	(3.01)	(1.58)	-	(1.41)	(1.48)	(1.23)	(0.75)	(0.65)	(1.22)	(0.66)	(1.56)	(1.00)	(1.05)	(1.59)	(1.01)	(1.65)	(0.73)	(1.04)	(3.53)
Stat sig. P <	-	0.05	-	-	-	-	-	0.05	-	-	-	-	0.05	-	-	-	0.05	-	-	0.05	-
Diabetic	0.17	2.13	3.87	5.05	-	1.85	1.86	3.55	2.75	2.00	4.60	-	7.53	-	3.60	8.58	5.88	5.37	6.34	3.52	5.58	23.25
	(0.23)	(0.74)	(1.09)	(1.10)	-	(0.65)	(1.08)	(1.31)	(0.37)	(0.49)	(0.62)		(1.25)		(0.65)	(1.72)	(2.20)	(0.89)	(0.72)	(0.66)	(1.74)	(5.33)
Nondiabetic	0.35	2.28	3.63	4.69	-	2.03	2.46	4.07	2.71	2.39	4.82	2.64	5.89	4.06	9.32	5.40	5.44	5.82	3.59	7.44	16.70
	(0.36)	(1.01)	(1.06)	(1.38)	-	(1.11)	(1.35)	(1.36)	(0.62)	(0.79)	(0.66)	(0.74)	(1.11)	(0.72)	(1.61)	(1.02)	(0.88)	(1.13)	(1.00)	(3.07)	(3.97)
Stat sig. P <	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005

TABLE 7.8 COMPARISON OF THE MEAN PERCENTAGE VALUES + 1 S.D. OF (a) THE W.S. FRACTIONS AND (b) THE U.S. FRACTIONS OF BROWN NUCLEAR CATARACTS OF ANY CORTICAL INVOLVEMENT FROM DIABETICS AND NON-DIABETICS.

		I	II			III							IV			V						VI
		1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
(a)	Diabetic	2.27 (1.40)	5.31 (1.53)	10.31 (2.79)	9.46 (1.81)	- -	3.24 (1.33)	6.16 (1.72)	4.68 (1.33)	3.79 (1.00)	2.87 (0.86)	7.52 (2.42)	2.74 (0.86)	7.41 (1.22)	3.56 (1.12)	7.85 (2.27)	3.70 (1.21)	2.27 (1.00)	1.60 (1.07)	0.71 (0.69)	0.82 (1.06)	12.20 (5.20)
	Non-diabetic	1.32 (1.23)	5.73 (1.79)	10.70 (2.89)	9.95 (1.97)	- -	3.58 (1.83)	5.40 (1.81)	5.67 (1.45)	3.67 (1.01)	2.67 (0.91)	7.80 (1.84)	2.59 (0.79)	7.13 (1.36)	4.02 (1.00)	7.38 (1.15)	3.56 (0.81)	2.03 (1.16)	1.69 (1.14)	0.95 (0.72)	0.98 (1.13)	10.91 (4.31)
	Stat sig. P<	-	-	-	-	-	-	0.05	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-
(b)	Diabetic	0.47 (0.68)	2.28 (1.28)	3.85 (1.79)	4.66 (1.64)	- -	2.22 (1.53)	2.36 (1.59)	3.30 (1.47)	2.52 (0.68)	2.56 (0.80)	4.60 (1.30)	2.39 (1.18)	5.60 (1.53)	3.54 (1.24)	8.74 (1.28)	5.43 (1.95)	5.83 (1.25)	5.60 (1.26)	4.32 (1.33)	8.61 (4.89)	16.80 (5.10)
	Non-diabetic	0.37 (0.48)	2.08 (1.26)	3.44 (1.44)	4.06 (1.58)	- -	1.93 (1.58)	2.26 (1.31)	3.34 (1.43)	2.31 (0.49)	2.31 (0.88)	4.20 (1.31)	2.71 (0.79)	5.56 (1.20)	3.90 (0.82)	9.28 (1.47)	5.50 (1.00)	5.96 (0.92)	6.17 (1.24)	4.20 (1.08)	12.41 (3.98)	16.29 (4.84)
	Stat sig. P<	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-

TABLE 7.9 COMPARISON OF MEAN PERCENTAGE VALUES ± 1 S.D. OF (a) THE W.S. FRACTIONS AND (b) THE U.S. FRACTIONS OF LENSES FROM DIABETIC PATIENTS WITH EITHER (i) BROWN NUCLEAR PLUS CUNEIFORM CATARACT OR (ii) BROWN NUCLEAR PLUS MATURE CORTICAL CATARACT WITH THE VALUES FOR LENSES OF SIMILAR MORPHOPATHOLOGY FROM NON-DIABETIC PATIENTS.

		I	II			III							IV			V						VI
		1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
(a) (i)	Diabetic	1.58 (1.41)	4.88 (0.59)	12.17 (2.31)	10.47 (2.25)	-	3.38 (1.68)	4.77 (2.08)	4.35 (1.13)	3.52 (1.94)	- 11.42 (3.96)	-	2.18 (1.68)	7.50 (0.44)	3.50 (1.11)	- 10.80 (0.70)	-	- 2.33 (1.40)	-	0.97 (0.87)	1.08 (1.13)		15.07 (4.69)
	Non-diabetic	1.30 (1.12)	6.19 (1.76)	10.64 (2.34)	10.34 (2.22)	-	3.68 (1.19)	5.56 (1.57)	5.64 (0.89)	4.18 (0.63)	- 9.96 (2.18)	-	3.06 (0.60)	6.25 (1.57)	3.97 (1.23)	- 10.88 (1.48)	-	- 2.58 (0.76)	-	0.85 (0.78)	0.79 (0.81)		13.22 (4.44)
	Stat sig. P <	-	-	-	-	-	-	-	0.005	-	-		-	-	-	-		-		-	-		-
(ii)	Diabetic	2.75 (1.15)	6.40 (1.37)	9.90 (3.02)	8.62 (2.52)	-	2.80 (1.51)	6.28 (1.85)	5.40 (1.30)	4.63 (0.93)	2.67 (0.06)	7.70 (1.89)	- 8.25 (2.08)	-	3.28 (1.72)	6.68 (2.05)	3.06 (0.84)	2.30 (0.80)	1.94 (0.92)	0.98 (0.68)	1.40 (1.41)		12.96 (6.69)
	Non-diabetic	1.14 (1.08)	4.72 (1.62)	10.45 (2.91)	8.99 (2.36)	-	3.07 (1.70)	4.13 (2.19)	5.72 (2.19)	3.14 (1.03)	2.51 (1.26)	8.96 (2.21)	- 9.66 (2.02)	-	4.24 (1.20)	7.31 (1.27)	4.16 (0.98)	2.53 (1.01)	1.73 (0.91)	1.04 (0.91)	1.27 (1.26)		11.75 (4.71)
	Stat sig. P <	0.0025	0.05	-	-	-	-	0.0005	-	-	-	-	-		-	-	0.025	-	-	-	-		-
(b) (i)	Diabetic	0.70 (1.21)	2.40 (1.70)	4.43 (1.45)	5.43 (1.15)	-	2.77 (0.78)	3.33 (1.18)	3.07 (1.86)	1.97 (0.67)	2.63 (0.55)	6.03 (1.96)	2.33 (1.51)	7.13 (3.87)	2.80 (1.22)	8.17 (1.03)	5.57 (2.50)	5.87 (0.68)	4.93 (1.81)	4.57 (1.00)	8.20 (4.90)		17.17 (3.58)
	Non-diabetic	0.24 (0.27)	2.30 (1.31)	3.87 (1.38)	4.19 (1.43)	-	2.48 (0.78)	2.85 (0.65)	3.85 (1.13)	2.43 (0.68)	2.14 (0.65)	4.19 (0.86)	2.98 (0.41)	5.21 (1.01)	3.96 (1.13)	9.06 (1.37)	5.06 (0.78)	5.78 (0.99)	6.35 (0.51)	4.01 (0.93)	14.73 (2.56)		16.61 (3.20)
	Stat sig. P <	-	-	-	-	-	-	-	-	-	-	0.005	-	0.05	-	-	-	-	0.005	-	0.0025		-
(ii)	Diabetic	0.39 (0.66)	3.03 (2.47)	4.31 (1.91)	4.33 (1.41)	-	2.31 (1.66)	2.45 (1.47)	4.54 (2.01)	2.96 (0.69)	2.49 (1.04)	4.14 (0.91)	3.03 (0.94)	5.34 (1.63)	4.22 (1.02)	8.61 (0.86)	5.00 (0.75)	5.44 (1.10)	5.60 (0.89)	4.00 (0.77)	10.19 (5.07)		17.24 (3.31)
	Non-diabetic	0.44 (0.53)	1.67 (0.98)	2.95 (1.26)	3.59 (1.40)	-	1.60 (0.81)	2.34 (1.08)	3.31 (1.26)	2.23 (0.69)	2.33 (0.80)	4.18 (0.97)	2.70 (0.66)	5.71 (1.05)	3.90 (0.74)	9.02 (1.41)	5.88 (1.16)	6.21 (0.82)	6.69 (1.29)	4.60 (0.94)	12.11 (3.73)		14.82 (4.94)
	Stat sig. P <	-	0.05	0.05	-	-	0.05	-	0.05	0.01	-	-	-	-	-	-	0.05	0.05	0.05	-	-		-

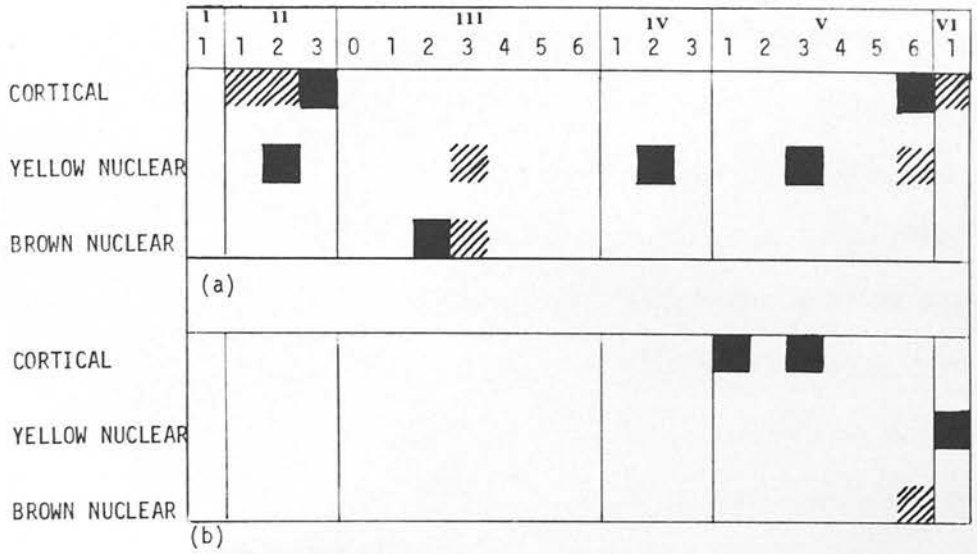


Fig. 7.3

Schematic representation of Tables 7.6, 7.7 and 7.8 indicating which sub units of (a) the W.S. fraction and (b) the U.S. fraction of diabetic lenses are present at significantly higher (■) or lower (▨) levels when compared to similar non-diabetic lenses. The lenses are grouped according to nuclear involvement regardless of the cortical morphopathology

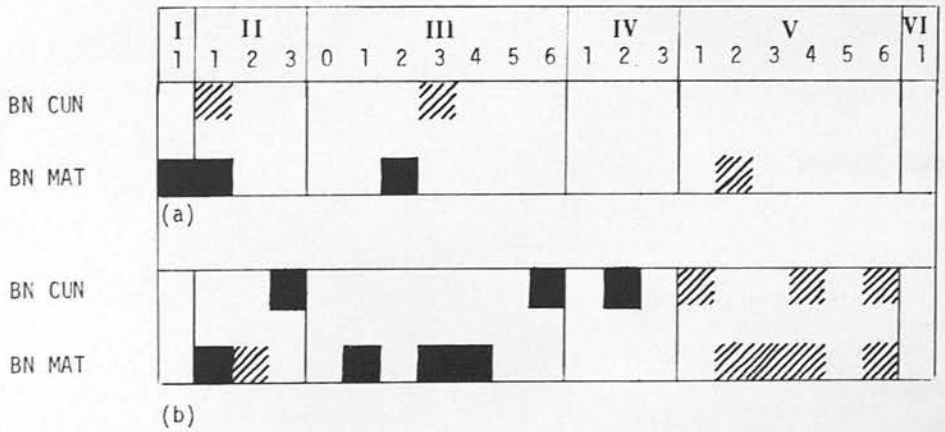


Fig. 7.4

Schematic representation of Table 7.9 indicating which sub units of (a) the W.S. fraction and (b) the U.S. fraction are significantly raised (■) or lowered (▨) in diabetic lenses with different cortical morphopathologies when compared with non-diabetic lenses of similar morphopathology. In this case the lenses considered are those with a brown nucleus plus cunei form (BN CUN) opacity and those with a brown nucleus plus mature cortical opacity (BN MAT).

group. This observation is an indication of how much information may be lost by the pooling of results. Differences which are characteristic of different stages of cataract may be obscured. However, some variation can be seen to be common to all diabetic cataracts. For example III_2 of the W.S. is high in both brown nuclear plus mature and in the total brown nuclear group. III_3 of the W.S. is low in both brown nuclear plus cuneiform and the total group of brown nuclear cataracts. V_6 of the U.S. is the total brown nuclear group and both of the subgroups mentioned. One phenomenon which is not illustrated by these results is the variation in subunit III_1 of the U.S. fraction. This subunit is either very low or undetectable in a significantly large number of diabetic individuals. But there is also a group of diabetics in whom this subunit is elevated. By pooling the diabetic together, these significant changes in the value of III_1 are lost.

Table 7.10.

Levels of subunit III_o of the U.S. fraction in diabetic and non-diabetic individuals.

% value of III_1	Diabetic	Non-diabetic	
0.5	11	33	44
0.5 - 3.9	28	399	427
4.0	5	30	35
	44	462	506

$\chi^2 = 17.33 \quad p < 0.0005$

2. Cataracts from Patients with Retinitis Pigmentosa.

Bellows and Bellows (1975b) include cataracts caused by retinitis pigmentosa in a group termed Cataracta Complicata. These cataracts are defined as occurring as a result of some other ocular disease and are divided into two groups; those resulting from diseases of the posterior segment and those from diseases in the anterior segment. Opacities in the posterior segment begin, usually, in the posterior cortex, but the authors do distinguish them from other posterior subcapsular cataracts with which they share some characteristics. Dilley, Bron and Habgood (1976) also aver that posterior subcapsular cataract is the typical change in retinitis pigmentosa but their microscopic studies suggested that the mechanisms of opacification showed similarities with those in senile cataract and aging lenses implying that there was one common mechanism which could be triggered by different stimuli. A further consideration is that of the genetic type of retinitis pigmentosa - dominant, recessive or sex-linked. Sorsby (1951) suggested that cataract is an expression of the dominant genetic disorder but that the association is less clear in the recessive form.

Ten cataractous lenses from patients with Retinitis Pigmentosa were obtained from various sources. Two of the lenses were from one patient. The information sent with these lenses was rather scant, except for three. Two of these three lenses had a posterior subcapsular cataract (one at least coming from a patient with dominant retinitis pigmentosa) and the other a brown nuclear plus early anterior subcapsular cataract. Because of the lack of detailed information it was difficult to compare the wet, dry and percentage dry weights with those of other groups of lenses. However,/

TABLE 7.11 MEAN PERCENTAGE VALUES ± 1 S.D. (IN BRACKETS) OF THE PROTEIN SUBUNITS OF THE W.S. AND U.S. FRACTIONS OF 10 LENSES FROM PATIENTS WITH RETINITIS PIGMENTOSA.

	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
W.S.	5.16 (1.74)	6.63 (1.08)	9.01 (0.78)	11.08 (1.15)	0.52 (0.58)	5.02 (1.19)	7.05 (0.91)	6.22 (0.48)	3.70 (0.43)	2.50 (0.76)	8.18 (0.72)	2.80 (0.54)	5.73 (0.65)	3.39 (0.21)	7.75 (0.83)	3.48 (0.74)	1.99 (0.45)	1.68 (0.52)	1.72 (1.16)	1.51 (1.10)	4.85 (1.78)
U.S.	4.56 (1.50)	3.47 (0.66)	5.02 (0.94)	6.70 (1.46)	1.35 (0.63)	3.31 (0.89)	3.65 (0.67)	4.21 (0.78)	2.78 (0.39)	2.09 (0.28)	5.07 (0.72)	2.72 (0.72)	4.20 (0.51)	3.31 (0.61)	7.71 (0.60)	4.73 (0.53)	5.02 (0.96)	5.83 (1.11)	3.21 (0.84)	8.55 (2.24)	11.77 (2.39)

TABLE 7.12 COMPARISON OF (a) THE W.S. FRACTIONS AND (b) THE U.S. FRACTIONS OF TWO GROUPS OF LENSES FROM RETINITIS PIGMENTOSA (R.P.) PATIENTS. ONE GROUP CONSISTS OF 7 LENSES, 3 OF WHICH HAVE SIMILAR MORPHOPATHOLOGY-MAINLY CUPULIFORM - AND ARE FROM PATIENTS WITH DOMINANT R.P., ALL SEVEN GIVING SIMILAR DENSITOMETRIC TRACES AND SUBUNIT INTEGRATION VALUES. THE OTHER GROUP CONSISTS OF 3 LENSES, ONE OF WHICH IS A BROWN NUCLEAR CATARACT COMING FROM A PATIENT WITH RECESSIVE R.P., WHICH GIVE DISSIMILAR DENSITOMETRIC TRACES AND INTEGRATION VALUES. THE FIGURES ARE MEAN PERCENTAGE VALUES ± 1 S.D. IN BRACKETS.

		I	II			III							IV			V						VI
		1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
(a)	7 cases	4.37 (0.75)	7.06 (0.88)	9.14 (0.91)	11.41 (0.86)	0.31 (0.55)	5.59 (0.72)	7.31 (0.89)	6.19 (0.35)	3.47 (0.25)	2.93 (0.37)	8.23 (0.43)	2.89 (0.62)	5.89 (0.38)	3.37 (0.15)	7.80 (0.71)	3.71 (0.24)	2.00 (0.42)	1.43 (0.39)	1.20 (0.78)	1.03 (0.60)	4.27 (1.62)
	3 cases	7.00 (2.17)	5.63 (0.91)	8.70 (0.26)	10.30 (1.57)	1.00 (0.30)	3.70 (1.04)	6.43 (0.75)	6.83 (0.72)	4.23 (0.21)	1.50 (0.20)	8.07 (1.33)	2.60 (0.26)	5.37 (1.10)	3.43 (0.35)	7.63 (1.26)	2.93 (1.29)	1.97 (0.60)	2.27 (0.21)	2.93 (1.04)	2.63 (1.27)	6.20 (1.55)
	Stat sig. P <	0.01	0.025	-	-	0.05	0.005	-	0.05	0.0025	0.0005	-	-	-	-	-	-	-	0.005	0.01	0.005	-
(b)	7 cases	5.27 (1.12)	3.44 (0.79)	5.01 (1.05)	6.89 (1.31)	1.38 (0.70)	3.51 (0.89)	3.79 (0.72)	4.30 (0.26)	2.79 (0.40)	2.19 (0.18)	5.09 (0.83)	2.63 (0.59)	4.41 (0.26)	3.01 (0.38)	7.87 (0.58)	4.80 (0.47)	5.06 (1.08)	5.66 (1.32)	2.93 (0.69)	7.60 (1.31)	11.23 (2.23)
	3 cases	2.90 (0.62)	3.53 (0.29)	5.03 (0.81)	6.27 (2.02)	1.27 (0.55)	2.83 (0.85)	3.20 (0.46)	4.00 (1.56)	2.77 (0.45)	1.87 (0.40)	5.03 (0.49)	2.60 (0.52)	3.70 (0.56)	4.00 (0.44)	7.33 (0.57)	4.57 (0.75)	4.93 (0.81)	6.23 (0.15)	3.87 (0.90)	10.77 (2.60)	13.03 (2.72)
	Stat sig. P <	0.005	-	-	-	-	-	-	-	-	0.05	-	-	0.025	0.005	-	-	-	-	0.05	0.025	-

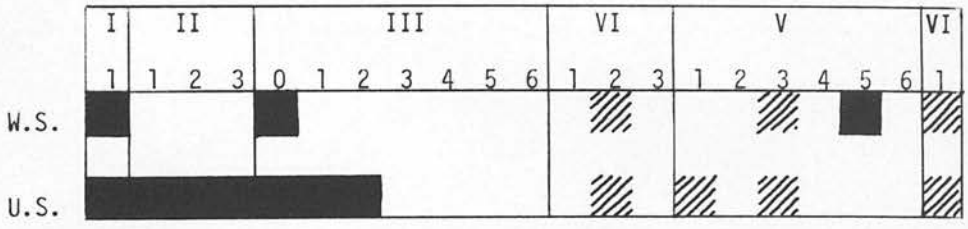


Fig. 7.5

Schematic representation indicating which sub units are relatively high (■) or low (▨) in the W.S. and U.S. fractions of lenses from patients with Retinitis Pigmentosa (R.P.) compared with the general population of cataractous lenses from the Edinburgh area.

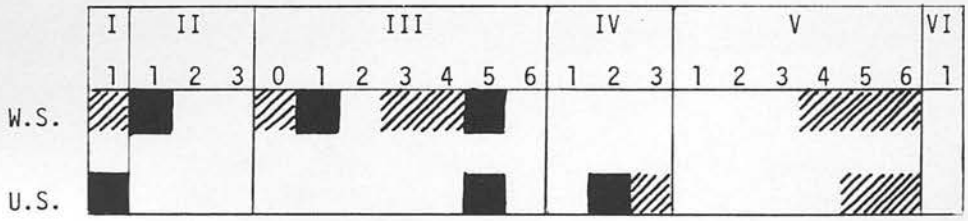


Fig. 7.7

Schematic representation of Table 7.12 indicating which sub units of the W.S. and U.S. fractions are significantly raised (■) or lowered (▨) in a group of seven R.P. lenses which give similar protein profiles compared with a group of three R.P. lenses which give protein profiles which are different from those of the group of seven.

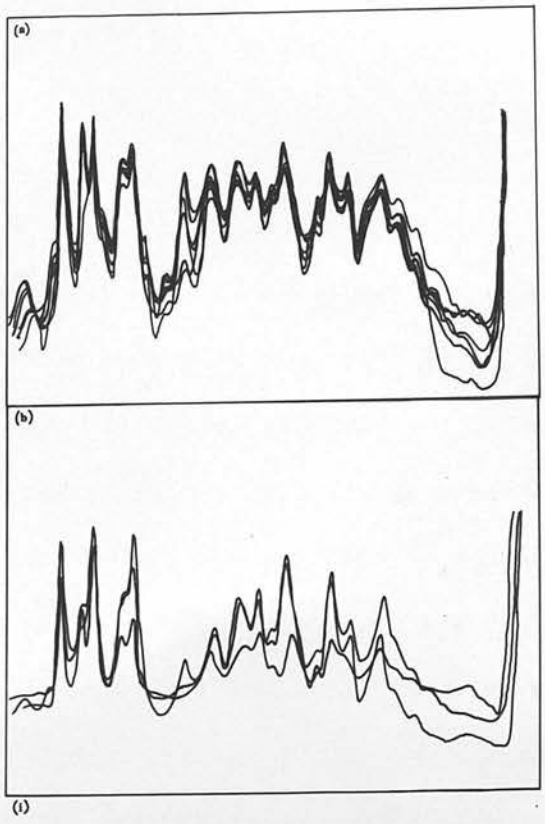
Fig. 7.6. (i) Superimposition of the densitometric traces of the W.S. fractions of (a) seven lenses from patients with retinitis pigmentosa (R.P.) which gave similar traces and (b) three lenses from R.P. patients which gave traces unlike those of the group of seven.

(ii) Average traces for the W.S. fractions of the two groups of lenses from the R.P. patients : (a) the group of seven and (b) the group of three.

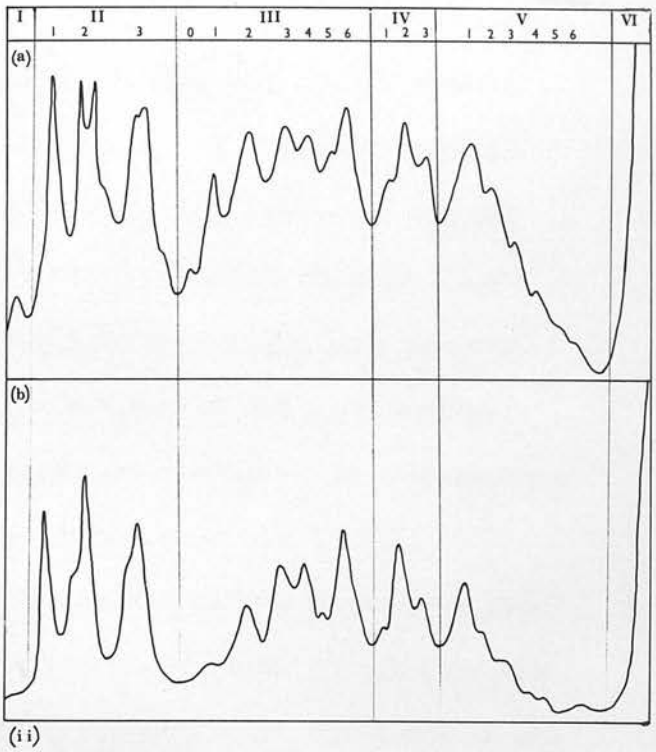
(iii) Superimposition of the densitometric traces of the U.S. fractions of the two groups of R.P. lenses described: (a) the group of seven and (b) the group of three.

(iv) Average traces for the U.S. fractions of the two groups of R.P. lenses : (a) the group of seven and (b) the group of three.

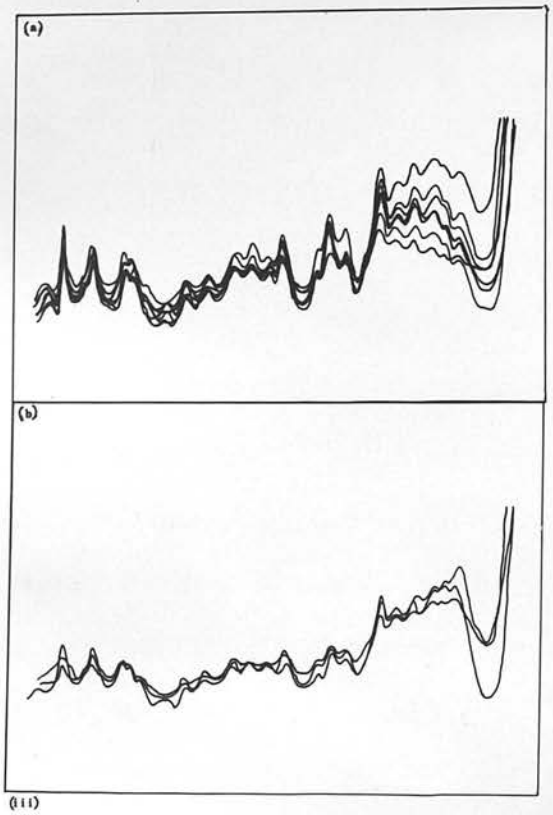
Grouping of the seven R.P. lenses was based on the similarity of their profiles while the other three were grouped as"the rest". This was the case since information regarding their morphopathology was absent in all but three cases. Two of the lenses had posterior polar subcapsular cataracts and were members of the group of seven. The third had an anterior polar subcapsular plus a brown nuclear cataract and was one of the group of three.



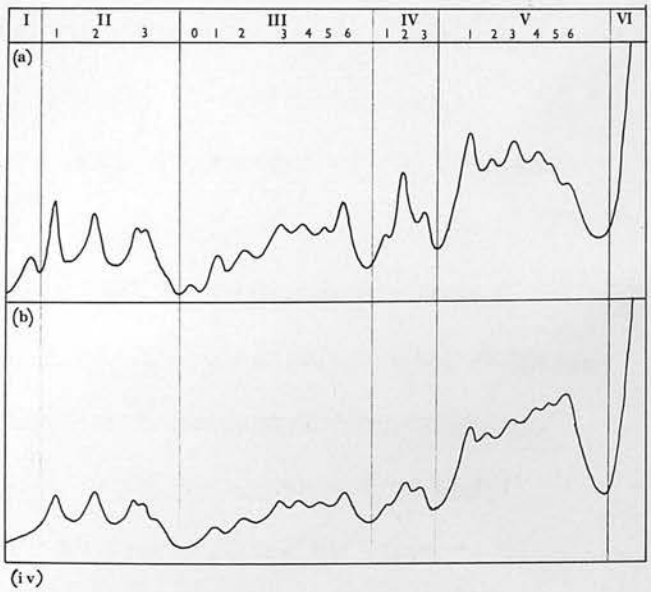
(i)



(ii)



(iii)



(iv)

Fig. 7.6

However, protein analysis by isoelectric focusing provided results which showed these lenses to be different in their protein profile from anything else. (Table 7.11 and Figure 7.5) Seven of the ten lenses including the two with posterior subcapsular cataract, produced remarkably similar traces and percentage values both for the W.S. and U.S. fractions. The remaining three including the brown nuclear plus anterior cortical cataract constituted a less homogeneous group. (Figure 7.6 and Table 7.12).

When the ten lenses were treated as one group and compared with non R.P. lenses (Table 7.11 and Figure 7.5) a number of features are apparent; first of all, the presence of subunit III_o which has rarely been observed in the general population of lenses and secondly high levels of I_1 and low levels of IV_2 V_3 and VI_1 in both the W.S. and U.S. fractions. These comparisons are relative rather than statistical and in relation to all the groupings considered in chapter six. A statistical comparison has been made between the group of seven similar profiles and the other three (Table 7.12) and the variations are illustrated in figure 7.7. If it is the case that the seven similar cataracts were the direct result of a similar type of R.P., then it may still be the case that the other three, while being different from the group of seven, are being affected, directly or indirectly, by the disease, since they still show differences from the general population of cataract lenses.

3. Cataracts from Leprosy Cases.

Thirty two cataractous lenses were obtained from a Korean leper colony. One other lens, was also obtained from a Korean who was not an ex-leper. They were transported in saturated ammonium sulphate solution. Because of this, no weight measurements could be made. These cataracts were examined in vivo and /

and morphopathological descriptions obtained. Twenty eight had a white nucleus and of those, twenty five had additional cortical involvement; 10 immature, 14 mature and 1 hypermature. The other five all had mature cortical involvement and varying nuclear involvement; 1 with yellow, 3 with brown and 1 with no nuclear colour (Table 7.13). The non-leper lens was white nuclear plus mature cortical. In addition to leprosy, or perhaps as a consequence of the disease, all the patients also suffered from uveitis and all were being treated with either atropine or hyoscine. Eleven patients were receiving cortico-steroid treatment. These factors may have had some additional or synergistic effect during cataractogenesis.

In making some general comparisons between the series of lenses from Edinburgh and the Korean leper lenses (whose average values are listed in table 7.14), there appears to be an inverse relationship between the W.S. and U.S. fractions. In the W.S. some subunits in groups I, II and III are high while those of groups V and VI are low. On the other hand in the U.S., some subunits in groups II and III are low while those of group V are high. Subunit IV_2 is relatively low in each case. When the groups of lenses with the following morphopathologies: white nucleus plus immature cortical involvement, and white nucleus plus mature cortical involvement are compared with lenses of similar morphopathology from Edinburgh the same pattern of difference is seen in each case (Tables 7.15 and 7.16 and Figures 7.8 and 7.9).

There is little difference however between the leper lenses with immature and mature cortical involvements (Figure 7.10). This is also the case, but to a greater degree, between lenses of similar morphopathologies from Edinburgh. /

TABLE 7.13 INCIDENCE OF CATARACT MORPHOPATHOLOGY IN CATARACTOUS LENSES FROM A KOREAN LEPER COLONY.

* INCLUDES ONE KOREAN LENS FROM A NON-LEPER.

CORTICAL INVOLVEMENT	NUCLEAR COLOUR				TOTALS
	NONE	WHITE	YELLOW	BROWN	
None	-	3	-	-	3
Immature	-	10	-	-	10
Mature	1	14*	1	3	19
Hypermature	-	1	-	-	1
Totals	1	28	1	3	33

TABLE 7.14 AVERAGE PROTEIN SUBUNIT PERCENTAGE VALUES (1 S.D. IN BRACKETS) OF THE W.S. AND U.S. FRACTIONS OF 32 CATARACTOUS LENSES FROM KOREAN LEPERS.

	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
W.S.	3.18 (2.89)	6.87 (1.41)	11.39 (3.11)	13.62 (2.38)	0.94 (1.06)	2.77 (1.48)	7.64 (1.27)	6.26 (1.14)	4.35 (0.67)	5.30 (2.89)	6.32 (1.77)	2.51 (1.16)	5.96 (1.17)	4.62 (0.81)	6.47 (1.59)	2.36 (0.90)	1.21 (0.93)	- 0.96 - (1.10)		0.34 (0.57)	6.26 (3.91)
U.S.	0.26 (0.22)	1.94 (0.82)	2.23 (1.08)	3.22 (1.11)	0.07 (0.12)	0.54 (0.44)	1.26 (0.65)	2.85 (0.77)	2.35 (0.63)	1.25 (0.35)	3.59 (0.75)	2.52 (0.48)	4.72 (0.81)	3.38 (0.74)	9.97 (1.16)	6.01 (0.48)	7.22 (1.58)	10.29 (1.84)	5.41 (0.75)	15.71 (4.02)	15.66 (3.40)

TABLE 7.15 COMPARISON OF THE PROTEIN SUBUNIT PERCENTAGE VALUES OF (a) THE W.S. FRACTIONS AND (b) THE U.S. FRACTIONS OF WHITE NUCLEAR PLUS IMMATURE CORTICAL CATARACTS FROM EDINBURGH (6 CASES) AND FROM A KOREAN LEPER COLONY (10 CASES). (AVERAGE VALUE PLUS 1 S.D. IN BRACKETS).

		I	II			III							IV			V						VI
		1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
(a)	Non-leper	2.32 (2.07)	5.37 (2.91)	8.80 (3.45)	9.77 (2.23)	- -	3.47 (1.82)	7.60 (2.26)	5.83 (1.42)	3.10 (0.66)	3.22 (0.34)	6.98 (1.17)	2.95 (0.67)	6.90 (1.98)	4.10 (1.19)	8.62 (2.06)	3.35 (0.79)	1.82 (0.74)	1.38 (0.57)	1.38 (0.84)	0.83 (0.54)	11.13 (6.78)
	Leper	2.23 (2.30)	7.07 (1.44)	10.12 (2.55)	13.42 (2.46)	1.00 (1.28)	3.06 (1.73)	7.87 (1.55)	6.84 (0.83)	4.28 (0.50)	3.25 (1.29)	7.77 (1.30)	3.48 (0.91)	5.93 (0.93)	4.89 (0.67)	7.23 (1.81)	2.61 (0.89)	1.40 (1.06)	- 0.74 - (0.77)		0.26 (0.44)	5.75 (4.30)
	Stat sig. $P <$	-	-	-	0.005	0.0001	-	-	0.05	0.0025	-	-	-	-	-	-	-	-	-		0.05	0.05
(b)	Non-leper	0.73 (0.93)	2.82 (1.92)	4.45 (2.34)	5.82 (3.00)	- -	2.25 (0.97)	2.28 (2.02)	3.22 (2.11)	2.06 (1.25)	2.22 (1.03)	4.55 (1.75)	2.42 (0.78)	5.42 (0.92)	3.42 (0.90)	9.50 (1.84)	5.73 (2.67)	6.05 (2.37)	5.95 (2.23)	4.42 (1.04)	6.80 (3.51)	19.72 (5.92)
	Leper	0.33 (0.25)	1.69 (0.53)	2.52 (1.06)	3.56 (0.01)	0.11 (0.19)	0.37 (0.25)	1.41 (0.57)	2.86 (0.51)	2.30 (0.72)	1.37 (0.18)	3.43 (0.82)	2.50 (0.48)	4.82 (0.81)	3.32 (0.93)	10.46 (1.59)	6.04 (0.64)	7.48 (1.31)	10.38 (2.41)	5.68 (0.68)	14.84 (3.46)	16.01 (2.85)
	Stat sig. $P <$	-	0.05	0.05	0.025	0.0001	0.0005	-	-	-	0.05	-	-	-	-	-	-	-	0.005	0.025	0.0025	0.05

TABLE 7.16 COMPARISON OF THE PROTEIN SUBUNIT PERCENTAGE VALUES OF (a) THE W.S. FRACTIONS AND (b) THE U.S. FRACTIONS OF WHITE NUCLEAR PLUS MATURE CORTICAL CATARACTS FROM EDINBURGH (5 CASES) AND FROM A KOREAN LEPER COLONY (13 CASES). (AVERAGE VALUES PLUS 1 S.D. IN BRACKETS).

		I	II			III							IV			V						VI
		1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
(a)	Non-leper	0.80 (0.73)	5.46 (3.73)	12.84 (7.08)	6.92 (2.95)	- -	2.90 (1.86)	3.70 (3.10)	5.05 (2.30)	3.27 (1.55)	2.46 (1.50)	5.93 (2.12)	2.17 (1.20)	5.33 (2.76)	3.34 (1.17)	7.80 (2.12)	4.00 (0.14)	- 4.30 - (1.67)		- 3.30 - (3.38)		22.76 (15.41)
	Leper	3.79 (3.09)	6.76 (1.57)	11.78 (3.76)	13.78 (2.34)	1.14 (0.87)	2.66 (1.28)	7.43 (1.25)	6.12 (1.20)	4.15 (0.91)	6.42 (3.21)	5.56 (1.77)	2.30 (0.95)	6.66 (2.01)	4.29 (1.05)	5.70 (2.40)	2.03 (0.89)	0.78 (0.91)	- 0.86 - (1.27)		0.27 (0.60)	7.12 (4.33)
	Stat sig. P <	0.05	-	-	0.0005	0.0001	-	0.0025	-	-	0.05	-	-	-	-	-	0.01	-	-	-	-	-
(b)	Non-leper	0.32 (0.52)	1.48 (0.91)	3.42 (1.67)	3.72 (1.56)	- -	1.80 (1.11)	1.84 (0.59)	3.50 (1.47)	2.46 (0.36)	2.02 (0.85)	3.74 (1.63)	2.45 (1.06)	5.85 (1.48)	3.80 (0.50)	8.64 (0.64)	6.36 (1.10)	6.52 (1.63)	5.84 (1.01)	4.14 (0.77)	8.24 (2.75)	24.82 (9.24)
	Leper	0.17 (0.18)	2.29 (0.91)	2.33 (1.13)	3.15 (1.40)	0.05 (0.07)	0.66 (0.49)	1.32 (0.75)	2.87 (0.91)	2.41 (0.67)	- 4.70 - (0.68)		2.60 (0.47)	4.85 (0.59)	3.20 (0.72)	9.99 (0.74)	6.01 (0.43)	7.48 (1.03)	10.67 (2.09)	5.28 (0.66)	14.54 (3.26)	15.28 (3.04)
	Stat sig. P <	-	0.05	-	-	0.0001	0.005	-	-	-	-	-	-	0.05	-	0.0025	-	-	0.0005	0.01	0.0025	0.0025

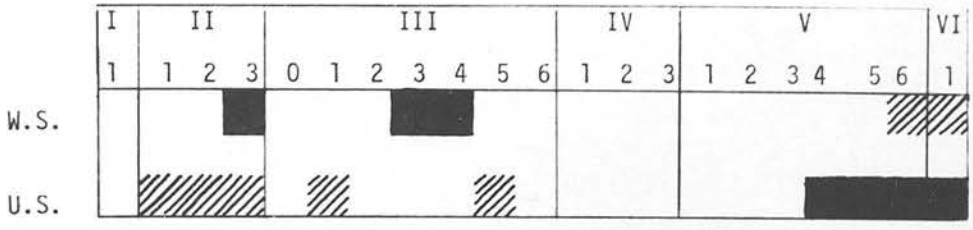


Fig. 7.8

Schematic representation of Table 7.15 indicating which sub units are significantly raised (■) or lowered (▨) in lenses from Korean ex-lepers. The W.S. and U.S. fractions of white nuclear plus immature cortical cataracts are compared with lenses of similar morphopathology from Edinburgh.

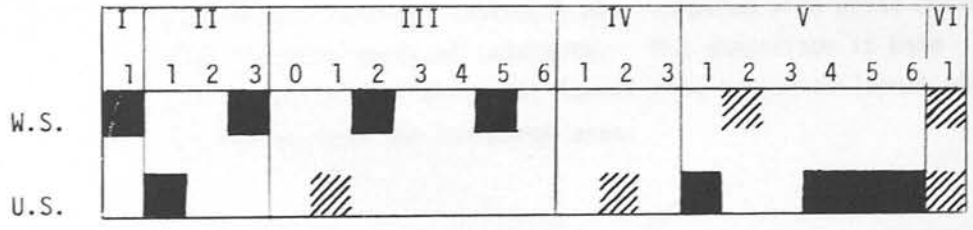


Fig. 7.9

Schematic representation of Table 7.16 indicating which sub units are significantly raised (■) or lowered (▨) in lenses from Korean ex-lepers. The W.S. and U.S. fractions of white nuclear plus mature cortical cataracts are compared with lenses of similar morphopathology from Edinburgh.

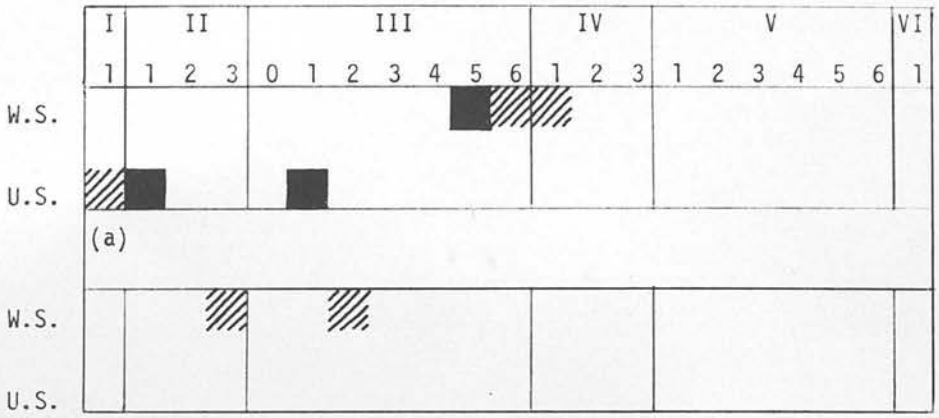


Fig. 7.10

Schematic representation indicating which sub units are significantly raised (■) or lowered (▨) in white nuclear plus mature cortical cataracts when compared with white nuclear plus immature cortical cataracts. The comparison is made within two populations; (a) lenses from Korean ex-lepers and (b) lenses from the Edinburgh area.

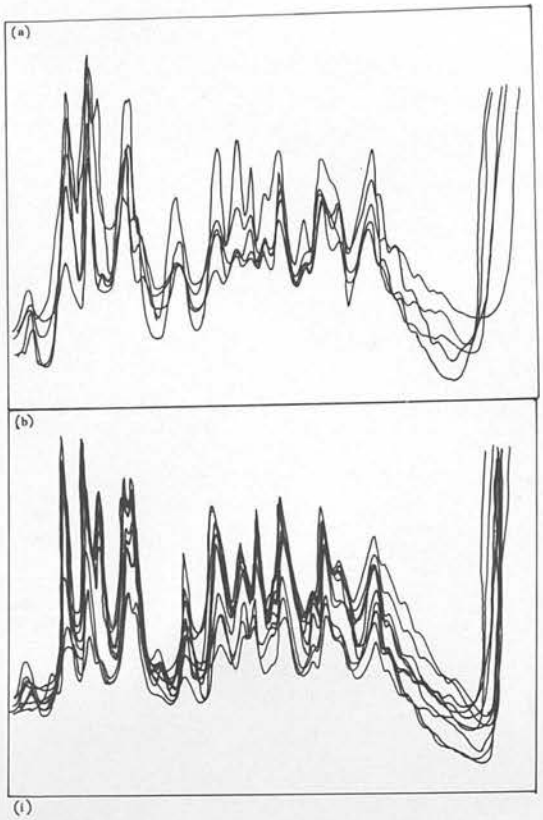
Fig. 7.11. (i) Superimposition* of densitometric traces of the W.S. fractions of white nuclear plus immature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

(ii) Average traces for the W.S. fractions of white nuclear plus immature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

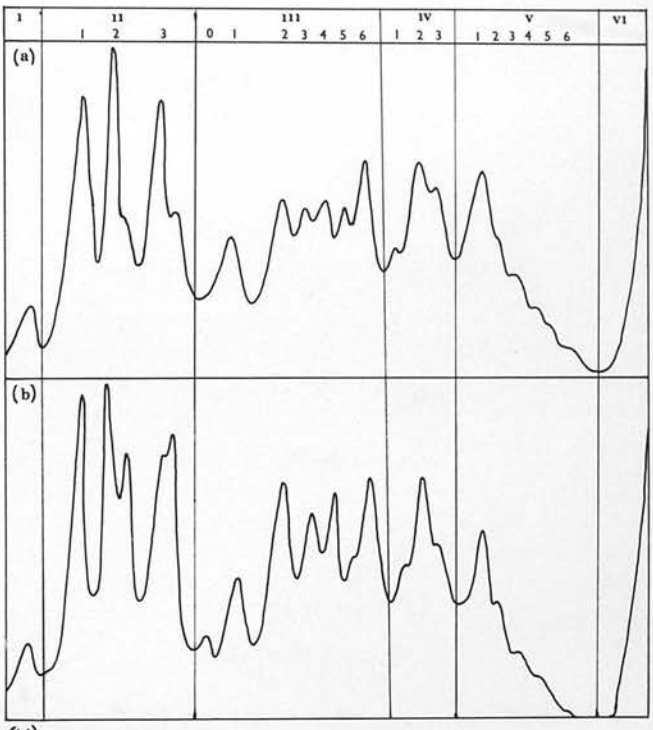
(iii) Superimposition* of densitometric traces of the U.S. fractions of white nuclear plus immature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

(iv) Superimposition* of densitometric traces of the W.S. fractions of white nuclear plus immature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

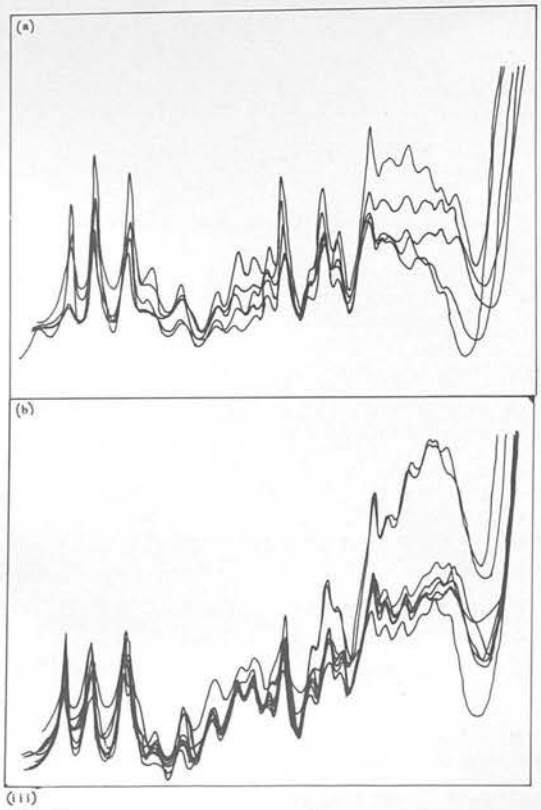
* While variation is expected within the groups of cataract analysed, the variation may appear exaggerated in this figure since some samples from different lenses were isofocussed on separate occasions. Because of this and the fact that the pH gradient may vary between gel runs, the peaks may take different slopes, sometimes high and narrow and other times low and broad. The areas, as integrated by the densitometer, show less variation; the range of the values for the profiles in fig. 7.11 can be seen in Table 7.15 and those for the profiles in fig. 7.12 in Table 7.16.


(a)
(b)

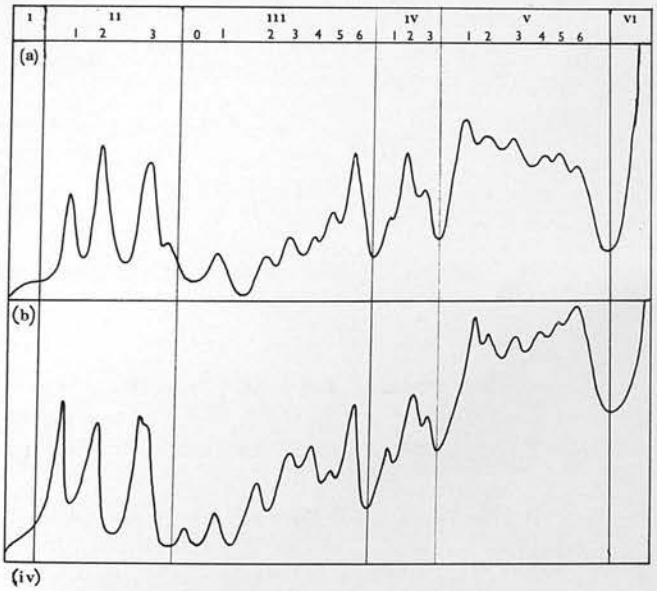
(i)


I
II
1 2 3
III
0 1 2 3 4 5 6
IV
1 2 3
V
1 2 3 4 5 6
VI
(a)
(b)

(ii)


(a)
(b)

(iii)


I
II
1 2 3
III
0 1 2 3 4 5 6
IV
1 2 3
V
1 2 3 4 5 6
VI
(a)
(b)

(iv)

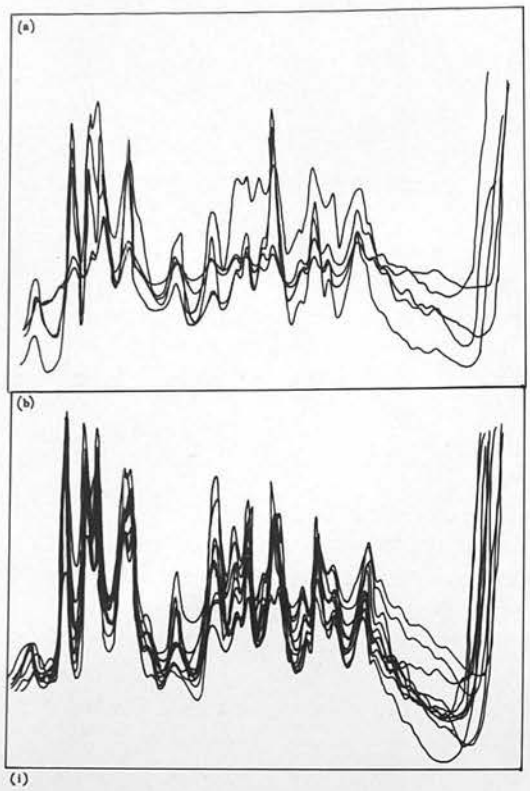
Fig. 7.12. (i) Superimposition * of the densitometric traces of the W.S. fractions of white nuclear plus mature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

(ii) Average traces for the W.S. fractions of white nuclear plus mature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

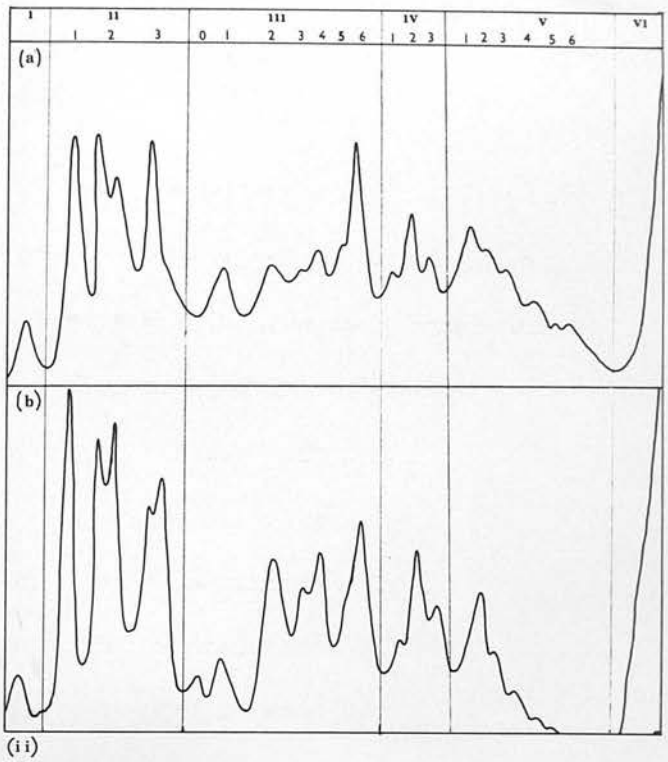
(iii) Superimposition * of the densitometric traces of the U.S. fractions of white nuclear plus mature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

(iv) Average traces for the U.S. fractions of white nuclear plus mature cortical cataracts from (a) the Edinburgh area and (b) a Korean leper colony.

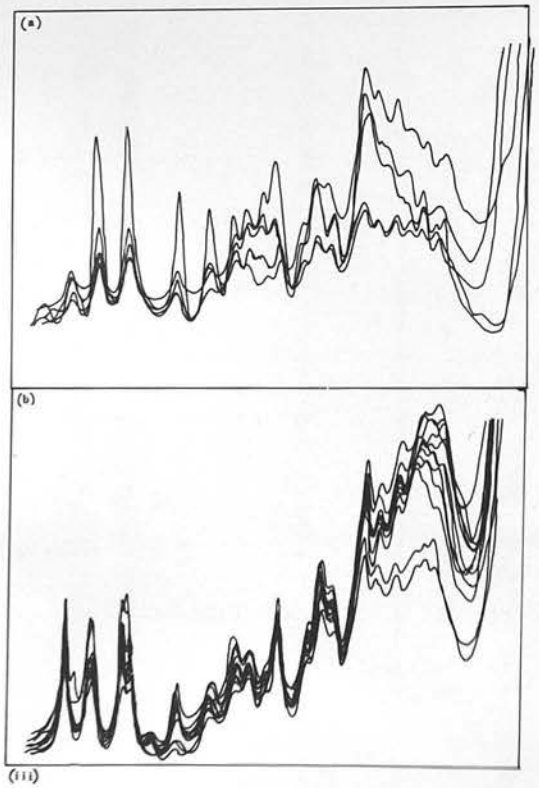
* While variation is expected within the groups of cataract analysed, the variation may appear exaggerated in this figure since some samples from different lenses were isofocused on separate occasions. Because of this and the fact that the pH gradient may vary between gel runs, the peaks may take different slopes, sometimes high and narrow and other times low and broad. The areas, as integrated by the densitometer, show less variation; the range of the values for the profiles in fig. 7.11 can be seen in Table 7.15 and those for the profiles in fig. 7.12 in Table 7.16.


(a)
(b)

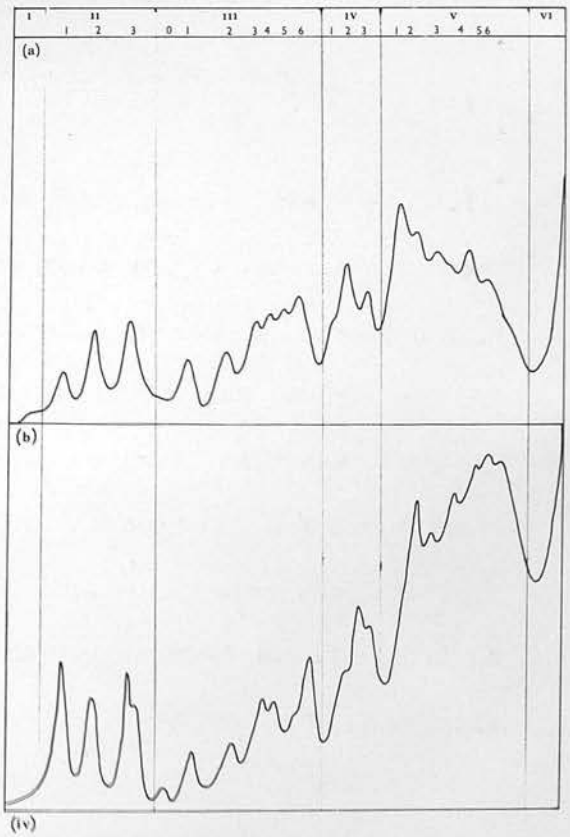
(i)


I
II
1 2 3
III
0 1 2 3 4 5 6
IV
1 2 3
V
1 2 3 4 5 6
VI
(a)
(b)

(ii)


(a)
(b)

(iii)


I
II
1 2 3
III
0 1 2 3 4 5 6
IV
1 2 3
V
1 2 3 4 5 6
VI
(a)
(b)

(iv)

TABLE 7.17 PROTEIN SUBUNIT PERCENTAGE VALUES OF THE W.S. AND U.S. FRACTIONS OF A WHITE NUCLEAR PLUS MATURE CORTICAL CATARACTOUS LENS FROM A KOREAN NON-LEPER.

	I	II			III							IV			V						VI
	1	1	2	3	0	1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	1
W.S.	0.2	3.1	6.2	6.0	0.0	1.7	1.9	5.0	4.3	-14.3-		3.4	-12.7-		10.0	4.9	4.4	3.4	1.6	2.1	14.3
U.S.	0.5	0.1	1.0	1.2	0.0	0.1	-2.1-		2.1	1.1	3.6	2.2	5.6	2.1	9.0	5.7	6.8	9.1	4.3	23.7	20.3

Fig. 7.13. Superimposition of the average traces for (a) the W.S. fractions and (b) the W.S. fractions of white nuclear plus mature cortical cataracts in lenses from the Edinburgh area (---) and from a Korean leper colony (·····) plus the single trace from a white nuclear plus mature cortical cataract from a Korean non-ex leper (—).

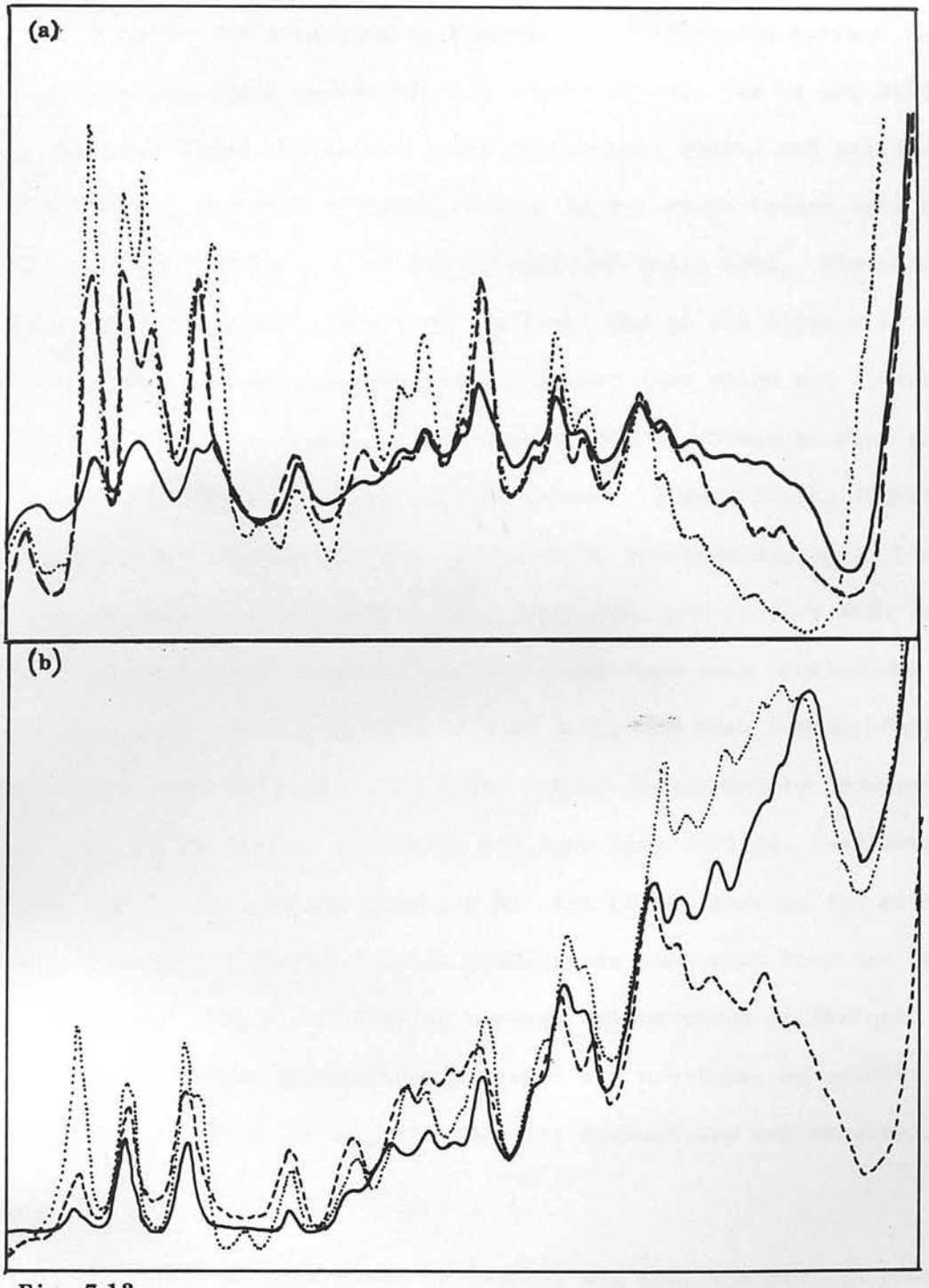


Fig. 7.13

from Edinburgh.

One question remaining is whether the differences between the leper and non-leper lenses are due to the disease, or to the difference in handling since the Korean leper lenses were stored and transported in saturated ammonium sulphate, while the Edinburgh lenses were frozen on extraction and stored in liquid nitrogen until used. The conclusion that some of the differences at least are due to the disease is supported by the results obtained from the one Korean lens which had a white nucleus plus mature cortical involvement and which was handled in exactly the same way as the leprosy lenses. These results (Table 7.12) show that the protein profile (in the W.S. fraction especially) of this lens resembles similar lenses from Edinburgh, and differs from lenses from Korean lepers. Further support comes from some preliminary findings - which will be no more than mentioned here - from analysis of lenses from India and Sri Lanka. These lenses mainly have brown and dark brown nuclear opacities and some have cortical involvements. They were treated in the same way as the Korean lenses, and although some differences in the protein profile are seen when they are compared to Edinburgh lenses, of similar nuclear colour these differences are not those observed to distinguish leper and non-leper cataracts: in other words, ammonium sulphate specific changes are not observed.

DISCUSSION.

While it has been shown in chapter six that the protein profiles of lenses differ with the location within the lens and with the stage of maturity of the opacity, the additional information of the presence of a disease has shown that even within a population of lenses, homogeneous/

homogeneous with regard to morphology, differences are present. Although it may be argued that, genetically, every lens must be different, statistical comparisons between groups establishes also that there is homogeneity within a group and, therefore, that the features of that group are a reflection of either the site of the lesion, or the effect of some cataractogen, or both. Moreover, the analysis of individual lenses before pooling the results allows grouping of the lenses at different levels. For instance, pooling of results to give the three populations: diabetic, R.P. and leprosy cataractous lenses, allowed the identification of some general trends characteristic of those populations respectively. However, splitting these populations to give smaller groups; in the case of diabetic lenses, those with brown nuclear plus cuneiform cataracts and those with brown nuclear plus mature cortical cataracts, and in the case of leper lenses, those with white nuclear plus immature cortical cataracts and those with white nuclear plus mature cortical cataract, allows a wider range of comparisons to be made. Comparisons can be made between lenses of similar aetiology but differing morphopathology or, alternatively between lenses of similar morphopathology but differing aetiology.

CHAPTER EIGHT

EPIDEMIOLOGY OF CATARACTS

It has been shown in the two previous chapters that if cataracts are grouped according to morphopathology, there are characteristics of the protein profiles which are indicative of that grouping, but on the other hand / a group of lenses characterised by the type of opacity need not necessarily be a homogeneous group. Such non-homogeneity is associated with differing aetiologies, as for example the case of diabetic cataracts, or cataracts from leprosy patients each of which groups shows a different value for certain subunits when compared to cataracts of similar morphopathology but different, (albeit unknown) aetiology.

In order to extend the value of this project, a group of people, including a scientist, a general practitioner, two ophthalmologists, a clinical chemist and a statistician, were brought together as a team to collect and analyse a formulated list of information for each individual in a population of patients and controls (Appendix A). The information collected included data on the patients' social background, medical history, some familial data, the present ophthalmological status, and clinical chemistry as screened by serum samples in addition to the biochemical results of the individual lens analyses already discussed here. The data were then analysed using the Statistical Package for the Social Science (S.P.S.S.) (Nie et al, 1975).

Single Factor Association

Analysis of data from the first 895 patients has shown a number of single factors to be significantly associated with cataract while some others show predominance in the control population.

There is a significantly increased level of cataract incidence in individuals with diabetes ($P<0.0001$), cardiovascular disease (especially in those individuals treated with antihypertensive drugs) ($P<0.002$), high blood pressure ($P<0.001$) and other eye diseases ($P<0.05$) (Tables 8.1 to 8.4). Also there is an increased incidence in individuals treated with major tranquilisers (barbiturates, MAOI, tricyclics and phenothiazines as opposed to the minor tranquilisers, valium and librium) ($P<0.05$) (Table 8.5). These drugs were grouped together to allow statistical comparisons to be made between small groups. With larger numbers, they will be considered individually. Drugs (including miotics and steroids) applied topically to the eye were used more frequently by the cataract population ($P<0.0005$) (Table 8.6) although there is no increase in individuals treated, either systemically or topically, with corticosteroids when they are considered separately as a group of drugs. There is, however, a lower incidence of cataract both in individuals who had suffered from serious infective bacterial illnesses ($P<0.005$) and those treated with antibacterial drugs ($P=0.005$) (Tables 8.7 and 8.8) and in individuals who are not total abstainers from alcohol ($P<0.0001$) (Table 8.9).

With respect to plasma constituents higher levels of urea and fasting plasma glucose ($P<0.0001$ in each case) are observed in patients while lower levels of calcium, cholesterol, total protein, albumin ($P<0.0001$ in each case), total CO_2 ($P<0.0005$), phosphate ($P<0.05$), alanine aminotransferase ($P<0.01$) and asparagine aminotransferase ($P<0.05$) are observed (Table 8.10).

Single factor assessment, while illustrating some significant association, has the disadvantage of not taking into account other relevant data. Two examples are blood pressure and fasting glucose levels in the plasma. The significance in the former may be affected by those in the population who are being treated with antihypertensive drugs, which are being used to control blood pressure, and in the latter by those in the population who are

overt diabetics although their glucose levels should be controlled eg. by a drug regime. In fact, when the necessary elements are removed from the total population, the significance remains (Tables 8.11 and 8.12) thus illustrating that individuals with high plasma glucose levels, whether they are overt or occult diabetics or not, are at risk, and also those with very high blood pressure. Those individuals who had received antihypertensive drugs, although in most cases their blood pressure was controlled, were still at risk, however (33 of the 658 patients and 1 of the 187 controls). This suggests that medication does not confer significant protection against cataract in this instance although at this stage it is difficult to determine whether cataract in this drug treated group is due to the complaint or the treatment.

Multifactorial Analysis

Since the total population for which data is available is relatively small, the subgroups which would be required for multifactorial analysis are, as yet, insufficient although it has been possible to carry out some analyses for a small number of parameters. The factors tested so far are four plasma constituents; glucose and urea, both of which increase the risk of cataract when their levels are raised, and urea and CO_2, both of whose levels are significantly higher in controls. The results show, in these cases, that when two risk factors (high glucose and high urea) are combined, there does seem to be a synergistic effect, 9.0% of patients as opposed to less than 1% of controls having both high (Table 8.13). When two "protective" factors (high cholesterol and high CO_2) are combined, the protection is at a level similar to high CO_2 alone which is a good deal less than with high cholesterol (Table 8.14). While high CO_2 appears to reduce protection when

combined with high cholesterol, it does not appear to reduce the risk when combined either with high glucose (Table 8.15) or high urea (Table 8.16). High cholesterol, however, balances the risk with high urea (Table 8.17) but only halves the risk with high glucose (Table 8.18). From these examples it can be seen that combinatorial analysis may be helpful in determining the interaction of risk factors and the possibility of positive or negative synergism.

Discussion

Epidemiological studies on cataract have been carried out throughout the world and from them various incidence rates have been proposed. However, the figures are not always comparable since the definition of cataract may vary between authors as well as the type of population studied. Caird et al (1965) found that in Oxford, one in 2,000 of the population over the age of twenty require cataract surgery, the rate increasing with age. Seventy-five per cent of the cases are classed as senile cataract. These figures agree with Sorsby's (1962) finding for England and Wales but are rather less than 4-7% after the age of 59 which was found by Brennan and Knox (1975) in Coventry. In India (Chatterjee, 1973) it was found that in the Punjab there was a higher rate of cataract at lower altitudes than in the Himalayas, the rates varying from 7.2% down to 1.2%. Chattergie also found that there was an earlier age of onset of cataract in India than in Western countries. Taylor (1980) found that cataract incidence in Australian Aborigines was significantly related to latitude, itself related to the number of hours of sunlight per day, and ultraviolet-B radiation levels. The average rate for the population studied was 33%. Such high rates have been found in other countries of similar latitude; 45% in Kenya (Bisley, 1963), 35% in Nigeria

(Osuntokun and Olurin, 1973) and 40% in India and Pakistan (Spector, 1974). In South Africa, Mann (1966) found cataract incidence to vary between ethnic groups; 10% in Europeans, 15-16% in Bantu and 33% in Indians.

Aetiological factors have also been studied. Both McGuinness (1967) and Caird (1973) found an increase in cataract surgery in diabetes although not of cataract itself. Axelson (1973) identified a number of drugs including demacarium and echothiophate in high levels, as cataractogens. While antibiotics have been found to be cataractogenic in two non-human systems - polymycin B in rats (Cotlier and Apple, 1976) and sulphonilamide in cultured lenses (Edwards et al, 1973) - in the present study, a higher prevalence of antibiotic use has been found in the control population. The Framingham Eye Study (Kahn et al, 1977 a and b) considered a local population who, thirty years previously, had been under investigation for coronary risk factors. They found an overall prevalence of senile cataract between ten and fifteen per cent and a significant association, in at least some age groups, between cataract and the following single variables; casual blood sugar (as opposed to fasting blood sugar in this study), systolic blood pressure (as opposed to diastolic blood pressure in this study), height, vital capacity, serum phospholipid and hand grip strength.

The present study has taken into account a large number of variables, some of which have already shown statistical significance, some of which, with larger numbers, may yet show statistical significance and others which almost certainly will be redundant. Some of the associations have agreed with the previous literature and others have conflicted. The identification of such associations will allow combinatorial investigations, which, one may hope, will lead to the identification of individuals at risk and the preventive measures which may be taken.

TABLE 8.1 INCIDENCE OF DIABETES

	Patients	Controls	Totals
Non-diabetics	619 (91.2%)	195 (100%)	814
Diabetics	60 (8.8%)	0 (0%)	60
Totals	679	195	874

$\chi^2 = 17.79$ $P < 0.0001$

TABLE 8.2 CARDIOVASCULAR CONDITION AND TREATMENT

	Patients	Controls	Totals
No Cardiovascular Condition	505 (73.5%)	166 (85.1%)	671
Untreated	28 (4.1%)	2 (1.0%)	30
Digitalis	36 (5.2%)	7 (3.6%)	43
Warfarin	1 (0.1%)	1 (0.5%)	2
Antihypertensives	34 (4.9%)	1 (0.5%)	35
Surgical	1 (0.1%)	2 (1.0%)	3
Other	53 (7.7%)	13 (6.7%)	66
Multiple	29 (4.2%)	3 (1.5%)	32
Totals	687	195	882

$\chi^2 = 22.83$ $P < 0.002$

TABLE 8.3 BLOOD PRESSURE AND CATARACT

	Patients	Controls	Totals
Very Low, 60 mm Hg	9 (1.4%)	6 (3.2%)	15
Median Range	556 (84.5%)	171 (91.4%)	727
Very High, 120 mm Hg	93 (14.1%)	10 (5.3%)	103
Totals	658	187	845

$\chi^2 = 14.01$ $P < 0.001$

TABLE 8.4 OTHER EYE DISEASES AND CATARACT

	Patients	Controls	Totals
No other eye disease	553 (84.2%)	159 (90.0%)	712
Other (specified)eye disease	104 (15.8%)	16 (9.1%)	120
Totals	657	175	832

$\chi^2 = 5.01$ $P < 0.05$

TABLE 8.5 USE OF PHYSCOACTIVE DRUGS

	Patients	Controls	Totals
None	561 (81.4%)	166 (85.1%)	727
Major	53 (7.7%)	5 (2.6%)	58
Minor	75 (10.9%)	24 (12.3%)	99
Totals	689	195	884

$\chi^2 = 6.71$ $P < 0.05$

TABLE 8.6 USE OF DRUGS TOPICALLY APPLIED TO THE EYE

	Patients	Controls	Totals
Non-use	618 (93.9%)	183 (99.5%)	801
Use	40 (6.1%)	1 (0.5%)	41
Totals	658	184	842

$\chi^2 = 9.58$ $P < 0.005$

TABLE 8.7 INCIDENCE OF CATARACT AFTER SERIOUS INFECTIVE BACTERIAL ILLNESS

	Patients	Controls	Totals
No SIBI	578 (75.8%)	126 (64.9%)	644
SIBI	165 (24.2%)	68 (35.1%)	233
Totals	683	194	877

$\chi^2 = 9.19 \quad P < 0.005$

TABLE 8.8 USE OF ANTIBIOTIC DRUGS

	Patients	Controls	Totals
Non-use	661 (96.6%)	182 (93.3%)	843
Use	23 (3.4%)	13 (6.7%)	36
Totals	684	195	879

$\chi^2 = 4.19 \quad P < 0.05$

TABLE 8.9 ALCOHOL CONSUMPTION

	Patients	Controls	Totals
Total abstention	293 (43.0%)	53 (27.2%)	346
Low occasional use	230 (33.7%)	104 (53.3%)	334
Moderate use	134 (19.6%)	28 (14.4%)	162
High use	17 (2.5%)	6 (3.1%)	23
Very high use	8 (1.2%)	4 (2.1%)	12
Totals	682	195	877

$\chi^2 = 28.23 \quad P < 0.0001$

TABLE 8.10 LEVELS OF PLASMA CONSTITUENTS IN CATARACT PATIENTS AND CONTROLS

Constituent	Patients		Controls		
	Mean	S.D.	Mean	S.D.	Significance
Urea (m mol/l)	61.92	22.22	54.45	13.60	$P < 0.0001$
Plasma fasting glucose (m mol/l)	55.42	20.70	47.49	4.99	$P < 0.0001$
Calcium (m mol/l)	238.93	14.78	245.49	10.07	$P < 0.0001$
Cholesterol (m mol/l)	61.66	14.17	66.60	12.06	$P < 0.0001$
Total protein (g/l)	69.66	6.11	73.18	4.51	$P < 0.0001$
Albumin (g/l)	40.72	4.10	43.09	2.44	$P < 0.0001$
Total CO_2 (m mol/l)	27.48	2.88	28.28	2.36	$P < 0.0005$
Phosphate (m mol/l)	9.61	6.48	10.82	9.69	$P < 0.05$
Alanine aminotransferase (U/l)	17.89	20.00	22.94	29.82	$P < 0.01$
Asparagine aminotransferase (U/l)	24.38	15.39	27.35	16.79	$P < 0.05$

TABLE 8.11 ASSOCIATION OF CATARACT AND BLOOD PRESSURE EXCLUDING INDIVIDUALS WITH HYPERTENSION

	Patients	Controls	Totals
Very low	9 (1.4%)	6 (3.2%)	15
Medium range	533 (85.3%)	171 (92.0%)	704
Very high	83 (13.3%)	9 (4.8%)	92
Totals	625	186	811

$\chi^2 = 13.6 \quad P < 0.001$

TABLE 8.12 PLASMA FASTING GLUCOSE IN NON-DIABETICS (DATA FROM AN EARLIER STAGE IN THE SURVEY)

	Patients	Controls	Totals
< 5.8 mmol/l	372 (86.7%)	99 (96.1%)	471
> 5.8 mmol/l	57 (13.3%)	4 (3.9%)	61
Totals	429	103	532

TABLE 8.13 COMBINATION OF TWO HIGH RISK FACTORS;
HIGH GLUCOSE AND HIGH UREA
(NORMAL INCLUDES THOSE INDIVIDUALS WITH LOW LEVELS)

	Patients	Controls	Totals
High Glucose and High Urea	55 (9.0%)	1 (0.8%)	56
High Glucose and Normal Urea	66 (10.8%)	3 (2.4%)	69
Normal Glucose and High Urea	151 (24.8%)	19 (15.0%)	170
Normal Glucose and Normal Urea	341 (55.6%)	104 (87.8%)	445
Totals	613	127	740

$\chi^2 = 34.63 \quad P < 0.0005$

TABLE 8.14 COMBINATION OF TWO LOW RISK FACTORS;
HIGH CHOLESTEROL AND HIGH CO_2
(NORMAL INCLUDES THOSE INDIVIDUALS WITH LOW LEVELS)

	Patients	Controls	Totals
High Cholesterol and High CO_2	31 (4.7%)	11 (5.7%)	42
High Cholesterol and Normal CO_2	78 (11.9%)	72 (37.3%)	150
Normal Cholesterol and High CO_2	54 (8.3%)	20 (10.4%)	74
Normal Cholesterol and Normal CO_2	491 (75.1)	90 (46.6%)	581
Totals	654	193	847

$\chi^2 = 73.02 \quad P < 0.0005$

COMBINATION OF HIGH AND LOW RISK FACTORS
(IN EACH CASE NORMAL INCLUDES THOSE WITH LOW LEVELS)

TABLE 8.15 HIGH GLUCOSE AND HIGH CO_2

	Patients	Controls	Totals
High Glucose and High CO_2	14 (2.1%)	0 (0.0%)	14
High Glucose and Normal CO_2	106 (16.3%)	4 (2.2%)	110
Normal Glucose and High CO_2	60 (9.2%)	10 (5.6%)	70
Normal Glucose and Normal CO_2	472 (72.4%)	166 (92.2%)	638
Totals	652	180	832

$\chi^2 = 34.51$ $P < 0.0005$

TABLE 8.16 HIGH UREA AND HIGH CO_2

	Patients	Controls	Totals
High Urea and High CO_2	33 (4.8%)	4 (2.6%)	37
High Urea and Normal CO_2	192 (27.9%)	26 (13.3%)	218
Normal Urea and High CO_2	56 (8.2%)	28 (14.4%)	84
Normal Urea and Normal CO_2	406 (59.1%)	138 (70.7%)	543
Totals	687	195	882

$\chi^2 = 24.08$ $P < 0.0005$

TABLE 8.17 HIGH UREA AND HIGH CHOLESTEROL

	Patients	Controls	Totals
High Urea and High Cholesterol	67 (9.9%)	14 (7.3%)	81
High Urea and Normal Cholesterol	157 (23.1%)	16 (8.3%)	173
Normal Urea and High Cholesterol	142 (20.9%)	71 (36.8%)	213
Normal Urea and Normal Cholesterol	313 (46.1%)	92 (47.6%)	405
Totals	679	193	872

$\chi^2 = 33.25$ $P < 0.0005$

TABLE 8.18 HIGH GLUCOSE AND HIGH CHOLESTEROL

	Patients	Controls	Total
High Glucose and High Cholesterol	37 (5.4%)	2 (1.0%)	39
High Glucose and Normal Cholesterol	83 (12.0%)	2 (1.0%)	85
Normal Glucose and High Cholesterol	172 (24.9%)	83 (42.8%)	255
Normal Glucose and Normal Cholesterol	398 (57.7%)	107 (55.2%)	505
Totals	690	194	884

$\chi^2 = 42.12 \quad P < 0.0005$

APPENDIX A

First three cards for collection and computer storage of information from the population of cataract patients and controls. Card 1 includes information on the individual's social background and general medical background, Card 2 includes information on the ophthalmological record of the individual and Card 3 includes information on the clinical chemistry of the individual. Each Card may carry up to 80 items each of which may be scored for a maximum of 12 alternatives.

Card Number 1 [1]

Case Number

Col. 2 3 4 5 6 7 8 9 10 11
No. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

Experiment Serial Number

Col. 12 13 14 15
No. [ ][ ][ ][ ]

Status/Patient Control Relative
1 2 3
16 [ ]

Year of Admission 17 [ ]
to Trial 18 [ ]

Month of Admission

1 Jan 7 July
2 Feb 8 Aug
3 Mar 9 Sept
4 Apr 10 Oct
5 May 11 Nov
6 June 12 Dec

19 [ ]

Date of Month 20 [ ]
of Admission 21 [ ]

Sex N/K Male Female
12 1 2
22 [ ]

23 [ ]
Age 24 [ ]

Age Group
12 N/K

1 0-9 6 50-59
2 10-19 7 60-69
3 20-29 8 70-79
4 30-39 9 80-89
5 40-49 0 90+

25 [ ]

Occupation Physical Hazard (specify)
11 NA. 12 NK 0 None
1 Trauma - particles (e.g.mining. lathe operator,quarrying)
2 Trauma, other (eg. diving)
3 Heat (eg. Foundry, glass blowing,) potter
4 Light (eg. Welding, microscopy)
5 Radiation, other (eg. microwave ovens, communications engineering, radar, X-ray)
9 Other, specify 26 [ ]

Occupation, Chemical Hazard (specify)
11 NA 12 NK 0 None
1 Organic solvents (paint, dry cleaner etc.)
2 Anaesthetist
3 Brewing/barman
4 Chemical manufacturing, petroleum
5 Fertiliser or crop spraying
6 Ashphalting, caulking, etc.
9 Other, spedify 27 [ ]

Occupation, Other hazard
11 NA 12 NK 0 None
1 Exposure to human infections
2 Exposure to animal infections
9 Other, specify 28 [ ]

Employment CODOT code

State occupation of Individual

29 30 31 32 33
NA.11. NK.12. [ ][ ][ ][ ][ ]

Status CODOT code

State occupation of head of household

34 35 36 37 38
NK.12 [ ][ ][ ][ ][ ]

Origin 12 NK
1 British Isles
2 Europe
3 Africa 15 years UK
4 " First generation
5 India 15 years UK
6 " First generation
7 Other (specify) 15 years in UK
8 As 7, first generation

39 [ ]

Card number 1 [1]

Experiment Serial Number 12 13 14 15

Status/Patient, Control Relative
1 2 3 16

Domicile. Location
12 NK
1 Edinburgh & Lothian
2 NW Scotland
3 NE "
4 SE "
5 SW "
6 Rest of Britain
7 Immigrant - recent
8 Immigrant > 15 years 40

Domicile, type
12 NK
1 Urban
2 Rural
3 Institutional/eg. asylum, prison, orphanage)
4 Services, merchant navy
5 Industrial estate (rural industrial)
6 No fixed address 41

Weight, Kg. 42 43 44

Height, cm. 45 46 47

MEDICAL HISTORY
Serious infective illness - Viral.
1. Yes Specify
0 None 11 NA 12 NK 48

Serious infective illness - bacterial
1 Yes Specify
0 None 11 NA 12 NK 49

Medical Conditions & treatment
Cardiovascular
11 NA. 12 NK 0 None 1 Untreated
2 Digitalis 8 Other, specify
3 Warfarin 9 Multiple
4 Antihypertensives
5 Surgical 50

Hepatic
11 NA. 12 NK. 0 None. 1 Untreated
2 Drug treatment (specify)
3 Surgical
4 Other, specify
9 Muktiple 51

Renal
11 NA 12 NK 0 None 1 Untreated
2 Drug (specify)
3 Surgical
8 Other (specify)
9 Multiple 52

Dermatological
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
3 Irradiation
8 Other (specify)
9 Multiple 53

Endocrinological EXCLUDING DIABETES
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
3 Irradiation
4 Surgery
8 Other (specify)
9 Multiple 54

Diabetes
11 NA 12 NK 0 None 1 Untreated
2 Diet alone
3 Oral hypoglycaemics
4 Insulin
9 Multiple 55

Age of onset of diabetes
11 NA 12 NK
0 0-9 years
1 10-19 years
2 20-29 years
3 30-39 years
4 40-49 years
5 50-59 years
6 60-69 years
7 70-79 years
8 80+ years 56

Card Number 1 [1]

Experiment Series Number 12 13 14 15 [ ]

Status/Patient Control Relative
1 2 3 16 [ ]

Rheumatoid
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
3 Surgery
8 Other, specify
9 Multiple 57 [ ]

Cancer
11 NA 12 UK 0 None 1 Untreated
2 Drugs (specify
3 Surgery
4 Irradiation
8 Other (Specify)
9 Multiple 58 [ ]

Immunological
11 NA 12 NK 0 None 1 Untreated
2 Drugs
8 Other (specify)
9 Multiple 59 [ ]

Mental & CNS
11 NA 12 NK 0 None 1 Untreated
2 Anticonvulsants
3 Surgery
8 Other (specify)
9 Multiple 60 [ ]

Respiratory
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
8 Other (specify)
9 Multiple 61 [ ]

Chronic Infection (specify)
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
8 Other (specify)
9 Multiple 62 [ ]

Psychiatric Status
(senile dementia)
11 NA 12 NK 0 None 1 Yes 63 [ ]

Drug history Regular use or more than 4 months continuous use

Tranquillisers
11 NA 12 NK 0 None
1 MAOI
2 Tricyclics
3 Phenothiazines
4 Barbiturates
5 Valium, Librium
8 Other, specify
9 Multiple 64 [ ]

Hypnotics
11 NA 12 NK 0 None
1 Mogodon
2 Choral hydrate
3 Paraldehyde
8 Other, specify
9 Multiple 65 [ ]

Analgesics
11 NA 12 NK 0 None
1 Aspirin
2 Codeine
3 Paracetamol
4 Phenacetin
8 Other, Specify
9 Multiple 66 [ ]

Antihistamines
11 NA 12 NK 0 None
1 Yes (specify) 67 [ ]

Hormones - excluding contraceptives, corticosteroids
11 NA 12 NK 0 NOne
1 Yes, Specify 68 [ ]

Oral Contraceptives
11 NA 12 NK 0 None
1 Yes (specify) 69 [ ]

Card Number 1 [1]

Experiment Serial Number 12 13 14 15

Status/Patient Control Relative
1 2 3 16

Drug History
Corticosteroids
11 NA 12 NK 0 None
1 Systemic (oral)
2 Systemic (injected)
3 Topical
9 Multiple 70

Antibiotics
11 NA 12 NK 0 None
1 Tetracycline
2 Frusemide
3 Isoniazid
4 Streptomycin
5 Sulphonamide
6 Penicilline
7
8 Other (specify)
9 Multiple 71

Vasodilators
11 NA 12 NK 0 None
1 Yes, Specify 72

Bronchodilators
11 NA 12 NK 0 None
1 Yes, specify 73

Diuretics
11 NA 12 NK 0 None
1 Yes, Specify 74

Alcohol (Daily average intake)
11 NA 12 NK 0 None
1 low, occasional use
2 Moderate (1-4 pints/2 doubles)
3 High use (5-10pints $\frac{1}{3}$ bot.)
4 Very high use (10 pints $\frac{1}{2}$ bottle daily) 75

Tobacco
11 NA 12 NK 0 None
1 Low, occasional
2 Moderate (10 cig./d. 1oz.)
3 High use (10-40/d.)
4 Very high use (40/d.) 76

Self-medication
11 NA 12 NK 0 None
1 Antacids
2 Laxatives
3 Cough mixtures
8 Other, Specify
9 Multiple 77

Reproductivehistory
11 NA 12 NK 0 None
1 1-4 children
2 5-10 "
3 Over 10 children 78

Age of menopause
11 NA 12 UK
1 Under 40
2 40-50
3 51-60
4 Over 61 79

Blood Pressure

11 NA 12 NK

B.P.: mm/Hg 80

Pre-medication before blood taken for clinical chemistry:

CATARACT ANALYSIS AND EPIDEMIOLOGY

Medical Record: Ophthalmological Record

Card Number 1 2

Experiment Number Serial 2 3 4 5

Status: Patient 1, Control 2, Relative 3 — 6

Familial Data
Numberof Ascertainable First Degree Relatives 7 8

Number of cataracts known or ascertained in first degree relatives. — 9

Number of ascertainable non Consanguineous relatives (spouse, adopted sibs, adoptive parents, step parent, adoptees.) — 10: 1

Number of Cataracts known or ascertained in non consanguineous relatives — 11: 0

Known Genetic Conditions of Individual:
Chromosomes NK 12, NA 11, None 0, Yes 1, (specify) — 12

Metabolism NK 12, NA 11, None 0, Yes 1, (specify) — 13

Skeleton NK 12, NA 11, None 0, Yes 1, (specify) — 14

Skin NK 12, Na 11, None 0, Yes 1, (specify) — 15

CNS NK 12, NA 11, None 0, Yes 1, (specify). — 16

Endocrines NK 12, NA 11, None 0, Yes 1, (specify) — 17

Eye NK 12, NA 11, None 0, Yes 1, (specify). — 18

For Patients under 10 years. Medical history of mother during pregnancy.

Infection NK 12, NA 11, None 0, Yes 1, (specify) — 19

Drugs NK 12, NA 11, None 0, Yes 1, (specify). — 20

Illness NK 12, NA 11, None,0, Yes 1, (specify) — 21

Other NK 12, NA 11, No 0, Yes 1, (specify) — 22

RIGHT EYE

Glaucoma NK 12, NA 11, No 0, OA 1, CA 2, Both 3, Secondary 4, — 23

Uveitis NK 12, NA 11, No 0, Anterior 1, Posterior 2, Both 3, — 24

Macular Degeneration NK 12, NA 11, No 0, Yes 1, — 25

Hypertensive Retinopathy NK 12, NA 11, No 0, Yes 1, — 26

Diabetic Retinopathy NK 12, NA 11, No 0, Yes 1, — 27

Retinal Detachment NK 12, NA 11, No 0, Yes 1, — 28

Physical Injury NK 12, NA 11, No 0, Perforating 1, Percussive 2, Radiation 3, Multiple 4, — 29

Other Eye Conditions (Buphthalmos) eg. (Microphthalmia) (Keratoconus) NK 12, NA 11, No 0, Yes 1, (specify) — 30

Aecus
slight 2
mod 3
Severe 4

Refraction before onset of cataract 31 32 33

Refraction at presentation 34 35 36

Years between First Record of Cataract and Operation
NK 12, NA 11, 0-1 0
2-5 1
6-10 2
11-15 3
16-20 4
21+ 5
37

Classification of Cataract (clinical appearance)
Nuclear
NK 12, NA 11, White 1, Yellow 2, Brown 3, Red/brown 4, Black 5.
38

Cortical- Anterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, multiple 9
39

-Posterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
40

-Peripheral
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
41

-Lamellar
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
42

-Sectorial
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
43

-Sutural
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
44

-Cuneiform
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
45

-Maturity Not Mature1
NK 12, NA 11, ~~Early 1~~, Mature 2, Hypermature 3,
46

Other
NK 12, NA 11, Blue dot 1, White dot 2, Coronary 3, bd + cor 4, bd + wd + cor 7, wd + cor 5, bd + wd 6
47

Distortion of Lens
Nk 12, NA 11,
Pseudoexfoliation 1,
Displacement 2,
48

LEFT BYE

Glaucoma NK 12, NA 11, No 0, OA 1, CA 2, Both 3, Secondary 4,
49

Uveitis NK 12, NA 11, No 0, anterior 1, posterior 2, both 3,
50

Macular Degeneration
NK 12, NA 11, No 0, Yes 1, (specify)
51

Hypertensive Retinopathy
NK 12, NA 11, No 0, Yes 1, (specify)
52

Diabetic Retinopathy
NK 12, NA 11, No 0, Yes 1, (specify)
53

Retinal Detachment
NK 12, NA 11, No 0, Yes 1,
54

Physical Injury
NK 12, NA 11, No 0, Perforating 1, ~~Percussive 2~~, Radiation 3, Multiple 4, Concussion 2.
55

Other Bye Conditions
(Buphtahlmos) (Microphthalmia) (Keratoconus)
eg.
NK 12, NA 11, No 0, Yes 1, (specify)
ARCUS slight 2 mod 3 Severe 4
56

Refraction Before Onset of Cataract
57 58 59

Refraction at Presentation 60 61 62

Years Between First Record of Cataract and Operation 63
NK 12, NA 11, 0-1 0
2-5 1
6-10 2
11-15 3
16-20 4
20+ 5

Classification of Cataract (clinical appearance)
Nuclear
NK 12, NA 11, White 1, Yellow 2, Brown 3, Red/Brown 4, Black 5. 64

Cortical -Anterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 65

-Posterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 66

-Peripheral
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 67

-Lamellar
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 68

-Sectorial
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 69

-Sutural
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 70

-Cuneiform
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4 Multiple 9 71

-Maturity
NK 12, NA 11, Early 1, Mature 2, Hypermature 3, 72

Other
NK 12, NA 11, Blue dot 1, White dot 2, Coronary 3, bd + cor 4, bd + wd + cor 7, wd + cor 5 bd + wd 6 73

Distortion of Lens
NK 12, NA 11, Pseudoexfoliation 1, Displacement 2, 74

Topical Applications of Drugs
NK 12, NA 11, None 0, Miotics 1, Adrenalin 2, Steroid 3, Other 4, (specify) 75

DATE:

Right Lens Left Lens.

CLINICAL CHEMISTRY

Card Number [1] [3]	Cholesterol [46] [47] [48]
Experimental Serial Number [2] [3] [4] [5]	Total Protein [49] [50] [51]
Plasma Analysis Urea [6] [7] [8]	Albumin [52] [53]
Sodium [9] [10] [11]	Triglycerides [54] [55] [56]
Potassium [12] [13]	Plasma Glucose, Fasting [57] [58] [59]
Total CO_2 [14] [15]	Plasma Glucose, 2h Post Glucose Load [60] [61] [62]
Bilirubin [16] [17] [18] [19]	Urine Analysis Urinary Cortisol/ Creatinine Ratio [63] [64] [65]
Alanine Aminotransferase [20] [21] [22] [23] [24]	
Alkaline Phosphatase [25] [26] [27] [28]	
Calcium [29] [30] [31]	
Phosphate [32] [33]	
Creatinine [34] [35] [36] [37]	
Urate [38] [39] [40]	
Aspartate Aminotransferase [41] [42] [43] [44] [45]	

CHAPTER NINE

CONCLUSIONS

It has been possible to classify a large number of human cataractous lenses, grouped according to nuclear colour and cortical morphopathology by their protein profiles. This has permitted a number of biochemically defined groups to be selected with sufficient numbers to allow statistical tests to be made on a number of parameters. Further, some groups of lenses with the same morphopathology could be subdivided on the basis of some known aetiological factors and it was found that a distinct aetiology was reflected by a distinct biochemical profile.

Cortical opacities were considered on the basis of their position within the lens and stage of maturity but without further subdivision according to the appearance of the opacity, that is whether there were water clefts, granules or vesicles. Such subdivision would have yielded too large a number of groups each consisting of too few cases, so that statistical analysis would be difficult. However, as this project continues, larger numbers will allow such comparisons to be made and, therefore, yield more information on the cataractogenic processes involved. In the meantime, it has been established that the term "senile cataract" is an unsatisfactory classification for a group of lenses, which are heterogeneous in many aspects, many of which may have associations with particular conditions and do not, therefore, represent an ageing process/

process as such.

The distribution of cataract types between the sexes was consistent and/not significantly different from the ratio of females to males (1.8 : 1) in the total population considered. Although figures are not available for the sex distribution with age in the Edinburgh area, the general observation that women live longer than men, and the observation made in this thesis that the male population had a significantly lower mean age than the female population, it might be suggested that women are not at greater risk than men despite the prediction of Weale (1979) that women are more liable to cataract than men due to increasing lens thickness with age.

The descriptions of the cataracts analysed in this study were made prior to extraction without any information on the duration or rate of progression of the cataracts. Differences in ages of onset or rate of maturations cannot, therefore, be assessed. However, white nuclear and cupuliform cortical opacities were shown to cause impairment of vision such that surgery was required at a significantly earlier age than with other forms of cataract.

Details of the age of onset and progression of cataract would be useful in the interpretation of the analysis of the wet, dry and percentage dry weights. It was shown in this analysis that the wet, dry and percentage dry weights must be related to the age of the lens at the time of cataract initiation rather than the age at the time of extraction. This is the case especially for cortical cataracts and, in particular, subcapsular opacities involving areas where there is active cell growth and protein synthesis. The balance/

balance of the water soluble : water insoluble protein must also be affected by the duration of the cataractogenic process.

Further subdivision of the selected cataract groups into age groups, is for the purposes of this study, of little use since this operation also, would yield too large a number of groups each with too few cases. However, in the future, when larger numbers would be available, such an operation would be useful since, apart from the various cataractogenic processes, there is a natural ageing process in which the protein profile of the lens changes due to the addition of newly synthesised material and also by post-synthetic modifications of proteins already present : neither of these processes necessarily lead to cataractogenesis although the latter may bring about a situation within the lens which makes it more susceptible to cataractogenic agents.

In the introduction, the evidence from the literature cited showed that there are a small number of identifiable direct gene products in each of the family of crystallins - α, β and γ - but, in addition there are a large number of modified gene products, this number increasing with age. From some preliminary results mentioned in this thesis, it is evident that the number of polypeptides present in the lens, as separated by 2-dimensional gel electrophoresis, is large and that some of them share similar isoelectric points or molecular weights. Thus, the number of bands distinguished by isoelectric focusing does not reflect the number of different polypeptide chains in the lens. Instead, some bands, separated in one dimension, may represent two, three or more polypeptides. However, given that some bands at least are not homogeneous/

homogeneous and, secondly, that not all of them have been characterised unambiguously with respect to its crystallin identification, the comparisons made between lenses are still valid, since the differences must be real regardless of the crystallin identification. Assessment would however be affected if cases were to occur where a band consisted of two constituent subunits and a decrease in one was offset by an increase in the other so that the percentage value of that peak on a densitometric trace remained unchanged.

However, all the data show that differences do occur between groups of lenses and that each classified group is uniquely characterised by the selection of subunits modified in amount, some being present at higher levels and some at lower levels than average. In general, the subunits which are provisionally identified as alpha-crystallin - or, at least, the peaks which described as containing alpha-crystallin - (II_2, II_3, III_6, VI_2 and V_1) exhibit variation with changes in the nuclear region and those peaks provisionally identified as - or containing - β-crystallin (II_1, II_2, II_3, III_1, III_2, III_3, III_4, III_5, IV_1 and IV_3) exhibit variation with changes in the cortex of the lens. These are general observations, however, and it is not a rule that only α-crystallins are affected in nuclear cataracts and only β-crystallins in cortical cataracts. The nature of the group V subunits has been tentatively described as cross-linked and aggregate material and since the amount of this material increases, in the U.S. fraction particularly, both with increasing cortical and increasing nuclear involvement, it might be suggested that cross-linked heavy molecular weight protein in/

in the human cataractous lens may contain both α- and β-crystallin; and since little γ-crystallin is found in either the W.S. or U.S. fractions at its usual isoelectric point (group I) it may be suggested that γ-crystallin too is involved in cross-linking and aggregation. It could be argued that involvement in aggregation is a more likely fate for γ-crystallin rather than leakage from the lens (it being a low molecular weight protein) since it is located mainly within the nucleus of the lens and, secondly, since it has a high sulphydryl content, a factor which is predisposing to covalent cross-linking.

Finally, a number of single variables have been associated with cataract but, again, these associations have been made on the basis of information collected at a single point in time. Therefore, the exact nature of the relationship, with respect to being either cause and effect, different symptoms of the same condition or totally independent but coincidental symptoms, is unclear in some cases. However, the degree of statistical significance of these associations would rule out the third option. The use of drugs associated with cataract would probably fall into the category of cause and effect as would some of the medical conditions such as diabetes and high blood pressure. There are, however, some situations where it may be uncertain whether it is the medical condition or the treatment which is the cause. With high blood pressure,for instance, the risk of cataract is increased and this risk is not decreased when the condition is controlled with antihypertensive drugs, which themselves are associated with cataract. The question remaining is/

is whether the initial risk of cataract with high blood pressure is not reduced even although the condition is controlled and whether the association of antihypertensive drugs with the incidence of cataract is fallacious or at least exaggerated. Alternatively they may both increase the risk and act synergistically. A similar example exists with glaucoma and the drugs used to treat it. Multifactorial analysis in the future, when numbers are large enough, will allow investigation of such possible synergistic interactions.

Levels of a number of blood plasma constituents have been found to differ significantly between patients and controls. Two constituents, urea and glucose, are both high in patients, the others which are significantly different being lower. The measurements were made just previous to cataract extraction and, therefore, the degree of cause and effect is uncertain. Monitoring the levels of constituents over a period of time during the initiation and progression of cataract would allow correlations to be made. However, their association with cataract is highly significant in each case and in some cases in agreement with previous findings in the literature. For instance, hypocalcaemia has been associated with cataract (Hanno and Weiss, 1961; Pohjola, 1962) while high levels of calcium in the lens have been found both in nuclear cataracts (Duncan and van Heyningen, 1976; Jedziniak et al. 1976). This inverse relationship might suggest that the lens might act as a sink in the body along with some other organs for some plasma constituents. Cholesterol follows a similar pattern to calcium in that there is an inverse relationship between levels in the blood and/

and levels in the lens. High serum levels appear to afford some protection against cataract. This finding, which may be unexpected, may have some support from the work of Rodriques and Krehl (1951), who found that a high fat diet reduced the freqeuncy in diabetic rats, and Heffley and Williams (1974) who found that the incidence of cataract in rats fed on a high galactose diet was reduced with increased levels of egg in the diet. But contradictory evidence was put forward by Bornstein and Nelson (1949) and Charalampous and Hegsted (1950) who found that a high fat diet brought about an earlier manifestation and increased the rate of progression of diabetic cataract in the rat. Hammar (1965), on the other hand, found that a high fat diet neither protected against nor increased the risk of cataract in diabetic rats.

Some preliminary findings using combinational analysis has shown that there is a synergistic effect when two high risk factors, high glucose and high urea levels, are combined. Also, the counteractive effect of combining high and low risk factors differs, counteraction in some cases resulting in reduced risk and, in others, resulting in removal of the risk.

Future studies will involve statistical analysis of cataract aetiological factors linked to the crystallin subunit analysis. In this way, not only the risk of cataract and the susceptibility of the lens to various cataractogenic agents will be found, but also the individual subunits which are affected in each case. This may help to elucidate the biochemical pathways involved in the development of cataracts of different aetiology but similar morpho-

pathology/

morphopathology since much is already known about the chemistry of the crystallins, for instance their amino acid composition, sulphydryl content and their susceptibility to post-synthetic modifications including cross-linking deamidation and glycosylation.

It is hoped that larger patient and control populations will allow multifactorial analysis so that combinations of factors which will reduce the risk of cataract may be identified. Identification of individuals who have some predisposition to cataract, whether genetic or environmental, might, in turn, allow preventive measures to be taken to at least reduce, if not remove, the risk of cataract in those individuals.

ACKNOWLEDGEMENTS

I am most grateful to my two supervisors, Mrs. R.M. Clayton and Professor C.I. Phillips for their advice and encouragement throughout the duration of this project and I would like to thank Dr. J.C. Campbell and Dr. D.E.S. Truman for useful discussion during both the project and the preparation of the manuscript. I also wish to thank Mr. A. Graham, Mr. F. Ramage, Mr. A. Gilchrist and Miss L.G. Hendrey, who provided technical assistance at various times, and all other members of the Edinburgh Lens Group who provided technical advice.

I am indebted to Professor C.I. Phillips, Dr. R.S. Bartholomew, Dr. J. Hughes and Dr. G.T. Millar of the Princess Alexandra Eye Pavilion, Edinburgh, who provided cataractous lenses for this study and Professor C.I. Phillips and Dr. R.S. Bartholomew in particular who carried out the clinical examinations and provided details of the cataract morphopathology. I am also grateful to Dr. N.L. Stokoe of the Royal Victoria Hospital, Kirkcaldy, who also provided lenses, and to Dr. A. Phillips and Dr. M. MacDonald who helped to transport these lenses to Edinburgh. I also wish to acknowledge the following surgeons who provided cataractous lenses from patients with retinitis pigmentosa; Mr. D.V. Ingram of the Sussex Eye Hospital, Mr. J.P. Travers, Noble's Isle of Man Hospital, Mr. D.J. Spalton, St. Thomas' Hospital, London, Mr. N.S.C. Rice and Mr. D.P. Greaves, Moorfields Eye Hospital, London, Mr. N.L. Stokoe, Royal Victoria Hospital, Kirkcaldy and Mr. T. Wilson, Falkirk District Royal Infirmary. Dr. Marshall and Mr. M. Boulton of the London Polytechnic helped in the collection and postage of some of the R.P. lenses. Mr. T. ffytche of St. Thomas' Hospital, London provided lenses from Korea for which I am grateful.

I wish to thank the members of the Edinburgh Cataract Epidemiology Study who made available the epidemiological information; Mrs. R.M. Clayton in the Department of Animal Genetics, Professor C.I. Phillips, Dr. R.S. Bartholomew, Dr. J. McKay Reid and Mrs. C. Wilson in the Department of Ophthalmology, Dr. J. Seth in the Department of Clinical Chemistry and Mr. J. Duffy in the Department of Statistics.

I wish to thank Miss M. Campbell, Miss M. Cuthbert and Miss M. McCutcheon who typed the final manuscript and Mrs. L. Dobbie who translated my writing into some of the first typed drafts. Mr. F. Johnston and Mr. J. Forrest carried out the photography, development and printing of most of the figures.

I am grateful to the Medical Faculty of the University of Edinburgh who provided me with a Whaitt Foundation Scholarship and to the Iris Fund for the Prevention of Blindness who provided me with a Research Associateship. I would like to thank Professor D.S. Falconer and Professor J.R.S. Fincham of the Department of Animal Genetics who made available the laboratory facilities.

Finally, but by no means least, I must thank my wife, Maria, for her patience and encouragement during this work.

REFERENCES

ADAMS, D. R. (1930) The Nature of Ocular Lesions Produced Experimentally by Naphthalene. Br. J. Ophthalmol. 14, 49-60.

ANTHONISEN, H. (1936) Frequency of Diabetic Cataract and Diabetic Glaucoma as compared to Frequency of Diabetes in General Population of Denmark. Acta. Ophthalmol. 14, 150-158.

ARAKI, M. and OKADA, T. S. (1978) Effects of Culture Media on the "Foreign" Differentiation of Lens and Pigment Cells from Neural Retina in vitro. Develop. Growth and Differ. 20, 71-78.

AREY, L. B. (1975) Development of the Lens. A Classical Approach. In Cataract and Abnormalities of the Lens. J. G. Bellows, Ed., Grune and Stratton, New York. pp.42-48.

AUGUSTEYN, R. C. (1979) On the Possible Role of Glutathione in Maintaining Lens Protein Sulphydryls. Exp. Eye Res. 28, 665-571.

AURICCHIO, G. and TESTA, M. (1972) Some Biochemical Differences Between Cortical (Pale) and Nuclear (Brown) Cataracts. Ophthalmologica, 164, 228-235.

AURICCHIO, G. and TESTA, M. (1973) Factors Affecting the Sulphydryl Groups of Structural Proteins of the Lens in Normal and "Uveitic" Eyes. Ophthal. Res. 5, 230-235.

AXELSSON, U. (1973) Miotic Induced Cataracts. The Human Lens in Relation to Cataract. Ciba Foundation Symposium 19. pp.249-257. Elsevier, Amsterdam.

BANDO, M., ISHI, Y. and NAKAJIMA, A. (1976) Changes in Blue Fluorescence Intensity and Coloration of Human Lens Protein with Normal Lens Ageing and Nuclear Cataract. Ophthal. Res. 8, 456-463.

BARBER, G. W. (1973) Human Cataractogenesis: A Review. Exp. Eye Res. 16, 85-94.

BARBER, G. W. (1974) Physiological Chemistry of the Eye. Arch. Ophthalmol. 91, 141-159.

BARTHOLOMEW R.S. (1980) – see paper at end of thesis

BECKER, O. (1877) Pathologic and Therapic des Linsensystems. In Handbuch der gesamten Augenheilkunde. Hrg. Graefe A. und Saemische E. T. Leipzig.

BELLOWS, J. G. (1944) The Crystalline Lens in Diabetes Mellitus. Arch. Ophthalmol. 32, 498-507.

BELLOWS, J. G. and BELLOWS, R. T. (1975) Cataracta Complicata. In Cataract and Abnormalities of the Lens. J. G. Bellows, Ed., Grune and Stratton, New York pp.272-275.

BELLOWS, J. G. and BELLOWS, R. T. (1975) Presenile and Senile Cataract. In Cataract and Abnormalities of the Lens. J. G. Bellows, Ed., Grune and Stratton, New York pp.303-313.

BENEDEK, G. B. (1971) Theory of Transparency of the Eye. Appl. Optics. 10, 459.

BERNDT (1834) cited by Bellows (1944

BETTELHEIM, A. F. (1975) On the Optical Anisotropy of Lens Fibre Cells. Exp. Eye Res. 21, 231-234.

BETTELHEIM, A. F. (1977) Induced Optical Anisotropy Fluctuation in the Lens of the Eye. J. Colloid Interface Sci., 63, 251-258.

BETTELHEIM , A. F. and WANG, I. J. Y. (1974) Topographic Distribution of Refractive Indices in Bovine Lenses. Exp. Eye Res. 18, 351-356.

BETTMAN, J. W., FUNG, W. E., WEBSTER, R. C., NOYES, P. P. and VINCENT N. J. (1968) Cataractogenic Effect of Corticosteroids in Animals. Amer. J. Ophthal. 65, 581-586.

BEYER-MEARS, A. and FARNSWORTH, P. N. (1979) Diminished Sugar Cataractogenesis by Quercetin. Exp. Eye Res. 28, 709-716.

BISLEY, G. G. (1963) The Prevention and Treatment of Blindness in Kenya. E. Afr. Med. J. 40, 570-573.

BJORK, I. (1961) Studies of γ-crystallin from Calf Lens. Exp. Eye Res. 1, 145-154.

BJORK, I. (1964)a Studies on the Subunits of α-crystallin and their Recombination. Exp. Eye Res. 3. 239-247.

BJORK, I. (1964)b Studies on γ-crystallin from Calf Lens. II Purification and Some Properties of the Main Protein Components. Exp. Eye Res. 3, 254-261.

BJORK, I. (1968) Comparative Studies of α-crystallin from Lenses of Different Mammalian Species. Exp. Eye Res. 7, 129-133.

BLACK, R. L., OGLESBY, R. B., VON SALLMAN, L. and BUNIM, J. J. (1960) Posterior Subcapsular Cataracts Induced by Corticosteroids in Patients with Rheumatoid Arthritis. J.A.M.A. 174, 166-171.

BLOEMENDAL, H., BERNS, H. J. M., VAN DER OUDERAA, F. and DE JONG W. W. (1972) Evidence for a "non-genetic" Origin of the A_1 Chains of α-crystallin. Exp. Eye Res. 14, 80-81.

BLOEMENDAL, H., BONT, W. S., JONGKIND, J. F. and WISSE, J. N. (1962) Splitting and Recombination of α-crystallin. Exp. Eye Res. 1, 300-305.

BLOEMENDAL, H. and HERBRINK, P. (1974) Growing Insight into the Structure of β -crystallin. Ophthal. Res. 6, 81-92.

BON, W.F. (1959) Rec. Trav. Chim. Pays-Bas 78, 75 cited by Bloemendal et al (1962).

BON, W. F. (1961) Physiochemical Investigations on a Complex Protein: the Soluble Protein of the Eye Lens, α-crystallin. J. Biol. Chem. 236, 81-85.

BONT, W. S., JONGKIND, J. F., WISSE, J. H. and BLOEMENDAL, H. (1962) The Effect of Urea on Lens Proteins. Biochem. Biophys. Acta 59, 512-514.

* **

BOURS, J. (1971) Isoelectric Focusing of Lens Crystallins in Thin Layer Polyacrylamide Gels. A Method for Detection of Soluble Proteins in Eye Lens Extract. J. Chromatogr. 60, 225-233.

BOURS, J. (1978) Isoelectric Focusing and Isotachophoresis of Rat Lens Crystallins in Dependence on Age. Interdiscipl. Topics. Geront. 12, 196-204.

BOURS, J., DOEPFMER, K. and HOCKWIN, O. (1976). Isoelectric Focusing of Crystallinsfrom Different Parts of the Bovine and Dog Lens in Dependence on Age. Docum. Ophthal. Proc. Ser. 8, 75-90.

BOURS, J., GRUBER, L., HOCKWIN, O. and HARRIS, J. (1978) The Crystallins of the Rat Lens with Triparanol-Induced Cataracts, also Related to Ageing. Interdiscipl. Topics Geront. 12, 127-131.

BOURS, J., WEBER, A. and HOCKWIN, O. (1976) The Crystallin Distribution of the Bovine Lens During Ageing. Les. Colloques de l'INSERM, 205-213.

*** see page 170

BOURS, J., WIECK, A. and HOCKWIN, O. (1978) Gel Filtration Chromatography of Crystallins and Nucleic Acids From Different Parts of the Bovine Lens in Dependence on Age. Interdiscipl. Topics Geront. 12, 205-220.

BOYLAND, E. (1963) Biochemistry of Bladder Cancer. p.35. Springfield Ill. Charles C. Thomas.

BRENNAN, M. E. and KNOX, E. G. (1975) The Incidence of Cataract and its Clinical Presentation. Community Health. 7, 13-20.

BUCKINGHAM, R. H. (1971) Cross-Links in Protein from Cataractous Lenses. Biochem. J. 124, 54P-55P.

BUCKINGHAM, R. H. (1972) The Behaviour of Reduced Proteins from Normal and Cataractous Lenses in Highly Dissociating Media: Cross-Linked Protein in Cataractous Lenses. Exp. Eye Res. 14, 123-129.

BURDITT, A. F. and CAIRD, F. I. (1968) Natural History of Lens Opacities in Diabetics. Br. J. Ophthalmol. 52, 433-440.

BURNS, A. T. H. (1975) RNA and Protein Synthesis in the Differentiation of the Lens. Ph.D. Thesis, University of Edinburgh.

BUSCHKE, W. (1943) Classification of Experimental Cataracts in the Rat; Recent Observations on Cataract Associated with Tryptophan Deficiency and with Some Other Experimental Conditions. Arch. Ophthalmol. 30, 735-750.

CAIRD, F. I. (1973) Problems of Cataract Epidemiology with Special Reference to Diabetes. Ciba-Foundation Symp. The Human Lens in Relation to Cataract. Associated Scientific Publications Amsterdam pp.281-296.

CAIRD, F. I., HUTCHISON, M. and PIRIE,A. (1964) Cataract and Diabetes. Br. Med. J. 2, 665-668.

CAIRD, F. I., HUTCHISON, M.and PIRIE, A. (1965) Cataract Extractions in an English Population. Brit. J. Prev. Soc. Med. 19, 80-84.

CAIRD, F. I., PIRIE, A. and RAMSELL, T. G. (1969) Diabetes and the Eye, Blackwell Scientific Publications, Oxford and London pp.127-162.

CAREY, H. U. and HUNT, H. M. (1935) Chemical Nature of Cataract in the Diabetic. N. Engl. J. Med. 212, 463-468.

CHAIKOFF, I.L. and LACKMANN, G.S. (1933) Occurrance of Cataract in Experimental Pancreatic Diabetes. Proc. Soc. Exp. Biol. Med. 31 237-241.

CHATTERJEE, A. (1973) Cataract in the Punjab. In, The Human Lens in Relation to Cataract. Ciba Foundation Symposium 19, 265-271.

CHYLACK, L. T. and KINOSHITA, J. H. (1969) A Biochemical Evaluation of a Cataract Induced in a High-Glucose Medium. Invest. Ophthalmol. 8, 401-412.

*** see page 170

CLARKE, R., ZIGMAN, S. and LERMAN, S. (1969). Studies on the Structural Proteins of the Human Lens. Exp. Eye Res. 8, 172-182.

CLAYTON, R. M. (1969) Properties of the crystallins of the Chick in Terms of their Subunit Composition. Exp. Eye Res. 8, 326-339.

CLAYTON, R. M. (1974) Comparative Aspects of Lens Proteins. In The Eye, eds., Davson, H. and Graham, L. T. Vol. 5, pp.399-494. Academic Press, New York.

CLAYTON, R. M., CAMPBELL, J. C. and TRUMAN, D. E. S. (1968) A Re-examination of the Organ Specificity of Lens Antigens. Exp. Eye Res. 7, 11-29.

CLAYTON, R. M. and TRUMAN, D. E. S. (1967) Molecular Structure and Antigenicity of Lens Proteins. Nature 214, 1201-1204.

COGAN, D. G., DONALDSON, D. D. and REESE, A. B. (1952) Clinical and Pathological Characteristics of Radiation Cataract. Arch. Ophthalmol. 47, 55-70.

COOPER, G. F. and ROBSON, J. G., (1969) The Yellow Colour of the Lens in Man and Other Primates. J. Physiol. 203, 411-417.

COTLIER, E. and APPLE, D. (1973) Cataracts Induced by the Polypeptide Antibiotic Polymyxin-B Sulphate. Exp. Eye Res. 16, 69-77.

COULOMBRE, A. J. (1965) Experimental Embryology of the Vertebrate Eye. Invest. Ophthal. 4, 411-419.

CREIGHTON, M. O., TREVITHICK, J. R., MONSA, G. Y., PEREY, D. H., McKINNA, A. J., DYSON, C., MAISEL, H. and BRADLEY, R. (1978) Globular Bodies: A Primary Cause of the Opacity in Senile and Diabetic Posterior Cortical Subcapsular Cataracts. Can. J. Ophthalmol. 13, 166-181.

***

CREWS, S. J. (1963) Posterior Subcapsular Lens Opacities in Patients on Long-Term Corticosteroid Therapy. Brit. Med. J. 1, 1644-1647.

CROFT, L. R. (1973) Low Molecular Weight Proteins of the Lens. In The Human Lens in Relation to Cataract. Ciba Foundation Symposium, Elsevier, Holland.

DAY, T. H. and CLAYTON, R. M. (1972) Multiple Changes in Lens Protein Composition Associated with the $Cat^{Fr.}$ Gene in the Mouse. Genet. Res. 19, 241-249.

de JONG, W. W., TERWINDT, E. C. and GROENEWOND, G. (1976) Subunit Composition of Vertebrate α-crystallins. Comp. Biochem. Physiol. 55B, 49-56.

DELCOUR, J. and PAPACONSTANTINOU, J. (1970) A Change in α-crystallin Subunit Composition in Relation to Cellular Differentiation in Adult Bovine Lens Biochem. Biophys. Res. Comm. 41, 401-406.

*** see page 170

DELCOUR, J. and PAPACONSTANTINOU, J. (1974) A Change in Stoichiometry of Assembly of Bovine Lens α-crystallin Subunits in Relation to Cellular Differentiation. Biochem. Biophys. Res. Comm. 57, 134-141.

DELCOUR, J. and BOUCHET, H. (1978) Evidence for a Post-Translational Origin of Subunit αB_1, in the Bovine Lens α-crystallin. Exp. Eye Res. 26, 191-195.

DILLEY, K. J., BRON, A. J. and HABGOOD, J. O. (1976) Anterior Polar and Posterior Subcapsular Cataract in a Patient with Retinitis Pigmentosa : a Light-microscopic and Ultrastructural Study. Exp. Eye Res. 22, 155-167.

DILLEY, K. J. and PIRIE, A. (1974) Changes to the Proteins of the Human Lens Nucleus in Cataract. Exp. Eye Res. 19, 59-72.

DISCHE, Z. (1968) Alterations of Lens Proteins as Etiology in Cataracts. In Bicohemistry of the Eye, Symp. Tutzing Castle pp.413-428. Karger, Basel/New York.

DISCHE, Z., ELLIOT, J., PEARSON, E. and MERRIUM, G. R. (1959) Changes in Protein and Protein Synthesis in Tryptophan Deficiency and Radiation Cataracts in Rats. Am. J. Ophthalmol. 47

DISCHE, Z. and ZIL, H. (1951) Studies in the Oxidation of Cysteine to Cystine in Lens Proteins During Cataract Formation. Amer. J. Ophthal. 34, 104-113.

DRENCKHAHN, D. and LULLMAN-RAUCH, R. (1977) Lens Opacities Associated with Lipidosis-like Ultrastructural Alterations in Rats Treated with Chloroquine, Chlorphentermine or Iprindole. Exp. Eye Res. 24, 621-632.

DUKE-ELDER, S. (1925) Changes in Refraction in Diabetes Mellitus. Br. J. Ophthalmol. 9, 167-187.

DUKE-ELDER, S. (1972) Toxic Cataract. In System of Ophthalmology, Vol. 11 105-119. Kimpton, London.

***

EDWARDS, A., GUPTA, J. D. and HARLEY, J. D. (1973) Photomicrophic Evaluation of Drug-induced Cataracts in Cultured Embryonic Chick Lens. Exp. Eye Res. 15, 495-498.

FRANCOIS, J. and RABAEY, M. (1957a) The Protein Composition of the Human Lens. Am. J. Ophthal. 44, 347-356.

FRANCOIS, J. and RABAEY, M. (1957b) On the Existence of an Embryonic Lens Protein. Arch. Ophthal. 57, 672-680.

FRANCOIS, J. and RABAEY, M. (1958) Permeability of the Capsule for Lens Proteins. Acta Ophthal. 36, 837-844.

FRANCOIS, J., RABAEY M. and RECOULES, (1961) A fluorescent Substance of Low Molecular Weight in the Lens of Primates. Archs. Ophthal. 65, 118-126.

*** see page 170

FRANCOIS, J., RABAEY, M. and STOCKMANS, L. (1965) Gel Filtration of the Soluble Proteins from Normal and Cataractous Human Lenses. Exp. Eye Res. 4, 312-318.

FRANCOIS, J., RABAEY, M. and WIEME, R. J. (1955) New Method for Fractionation of Lens Proteins. Arch. Ophthal. 53, 481-486.

FRIEDBURG, D., KRONER, M. and ROSENSTIEL, L. (1974) In vivo Action of Corticosteroids on the Guinea Pig Lens. Ophthal. Res. 6, 131-137.

FUKUI, S. and YAMASHINA, I. (1978) Biochemical Alterations of the Lens Capsule in Mice with Hereditary Cataract. Exp. Eye Res. 26, 499-506.

FULHORST, H. W. and YOUNG, A. W. (1966) Conversion of Soluble Lens Proteins to Albuminoid. Invest. Ophthal. 5, 298-303.

GABBAY, K. H. and KINOSHITA, J. H. (1972) Mechanism of Development and Possible Prevention of Sugar Cataracts. Isr. J. Med. Sci. 8, 1557-1561.

GALLUS, E. (1919) Gibt es eine Cataracta Diabetica? Deutsche Med. Wchnschr. 45, 658-659.

GIBLIN, F. J. and REDDY, V. N. (1978) High Molecular Weight Protein Aggregates in X-ray Induced Cataract : Evidence for the Involvement of Disulphide Bonds. Interdiscipl. Topics Geront. 12, 94-104.

GILES, C. L., MASON, G. L., DUFF, I. F. and McLEAN, J. A. (1962) The Association of Cataract Formation and Systemic Corticosteroid Therapy. J.A.M.A. 182, 719-722.

GOLDMANN, H. (1929) Experimentalle Supranuklearkataract und Kernsklerose. Klin. Mb. Augenhilk. 83, 433-438.

GORDON, B. L. (1949) Effect of Diabetes on Cataract and on Vision. Arch. Ophthal. 41, 450-459.

GRAY, W. A. (1933) The Ocular Conditions in Diabetes Mellitus. Brit. J. Ophthalmol. 17, 577-620.

GREINER, J. V. and CHYLACK, L. T. (1979) Posterior Subcapsular Cataracts: Histopathologic Study of Steroid-Associated Cataracts. Arch. Ophthalmol. 97, 135-144.

***
***
***

HARDING, J. J. (1972) Conformational Changes in Human Lens Proteins in Cataract. Biochem. J. 129, 97-100.

HARDING, J. J. (1976) Two Themes of Ageing Related to Changes in Lens Proteins. Colloque de l'INSERM. Courtois, Y. and Regnault, F. Eds. pp.215-222.

HARDING, J. J. (1979) Aggregation of Proteins in Human Cataract. Ophthal. Res. 11, 429-432.

*** see page 170

HARDING, J. J. and DILLEY, K. J. (1976) Structural Proteins of the Mammalian Lens: a Review with Emphasis on Changes in Development, Aging and Cataract. Exp. Eye Res. 22, 1-73.

HARRIS, J. E. and GRUBER, L. (1969) The Reversal of Triparanol Cataracts in the Rat. Documenta Ophthal. 26, 324-333.

HARRIS, J. E. and GRUBER, L. (1972) Reversal of Triparanol-induced Cataracts in the Rat. II. Exchange of ^{22}Na, ^{42}K and ^{86}Rb in Cataractous and Clearing Lenses. Invest. Ophthalmol. 11, 608-616.

HARRIS, J. E. and GRUBER L. (1973) Reversal of Triparanol-induced Cataracts in the Rat. III Amino-acid Content and uptake of ^{14}C -AIB in Cataractous and Clearing Lenses. Invest. Ophthalmol. 12, 385-388.

***

HERBRINK, P. and BLOEMENDAL, H. (1974) Studies on -crystallin: Isolation and Partial Characterisation of the Principal Polypeptide Chain. Biochim. Biophys. Acta. 336, 370-382.

HINES, M. C. and OLIVE, J. A. (1970) On the Components of Rabbit Lens ɣ-crystallin. Life Sci. (Biochemistry) 9, 1101-1110.

HOCKWIN, O. (1976) Aging of the Lens. Colloque de l'INSERM. 263-279.

HOCKWIN, O., BECHTEL-EHRIG, V., LICHT, W., NOLL, E. and RAST, F. (1971) Concerning the Estimation of the Age of Guinea Pigs, Rabbits and Chickens by Means of Determining their Lens Weight. Ophthal. Res. 2, 77-85.

*** see page 170

HOCKWIN, O. and KOCH, H-R (1975) Combined Noxious Influences. In Cataract and Abnormalities of the Lens, Ed. J. G. Bellows, Grune and Stratton, New York, pp.243-254.

HOENDERS, H. J., VAN KAMP, G. J., LIEM-THE, K. and VAN KLEEF, F. S. M. (1973) Heterogeneity, Aging and Polypeptide Composition of -crystallin from Calf-lens. Exp. Eye Res. 15, 193-200.

HOLLWICK, F., BOATENG, A. and KOLCK, B. (1975) Toxic Cataract. In Cataract and Abnormalities of the Lens. Ed. Bellows, J. G. Grune and Stratton, New York, pp.230-243.

IKEMOTO, F. and IWATA, S. (1978) Sulphydryl Contents of Soluble and Insoluble Lens Proteins in Naphthalene and Traumatic Cataracts in Rabbits. Ophthal. Res. 10, 194-201.

IRVINE, W. J. (1977) Classification of Idiopathic Diabetes. Lancet, March, 1977 VolI 638.

JEDZINIAK, J. A., BARAM, H. and CHYLACK, Jr., L. T. (1978) The Molecular Distribution, Weight Determination and Concentration Variation of the Total Water Soluble Proteins of the Human Lens. Exp. Eye Res. 26, 377-388.

JEDZINIAK, J. A., KINOSHITA, J. H., YATES, E. M. and BENEDEK, G. B. (1975) The Concentration and Localisation of Heavy Molecular Weight Aggregates in Aging Normal and Cataractous Human Lenses. Exp. Eye Res. 20, 367-369.

JEDZINIAK, J. A., KINOSHITA, J. H., YATES, E. M., HOCKER, L. O. and BENEDECK, G. B. (1972) Calcium-induced Aggregation of Bovine Lens Alpha-crystallins. Invest. Ophthal. 11, 905-915.

JEDZINIAK, J. A., KINOSHITA, J. H., YATES, E. M., HOCKER, L. O. and BENEDEK ., G. B. (1973) On the Presence and Mechanism of Formation of Heavy Molecular Weight Aggregates in Human Normal and Cataractous Lenses. Exp. Eye Res. 15, 185-192.

KABASAWA, I., BARBER, G.W. and KINOSHITA, J.H. (1977) Lens Low Molecular Weight Proteins. Jap. J. Ophthal. 21 87-97.

KABASAWA, I., TSUNEMATSU, Y., BARBER, G. W. and KINOSHITA, J. H. (1977) Low Molecular Weight Proteins of the Bovine Lens. Exp. Eye Res. 24, 437-448.

KERN, R., ZARUBA, K. and SCHEITLIN, W. (1970) Ocular side-effects of Longterm Immunosuppressive Therapy in Recipients of Cadavar Kidney Transplants. Ophthal. Res. 1, 21-30.

*

**

KINOSHITA, J. H. (1965) Cataracts in Galactosaemia. Invest. Ophthalmol. 4, 786-799.

KINOSHITA, J. H. (1974) Mechanisms Initiating Cataract Formation. Invest. Ophthalmol. 13, 713-724.

KINOSHITA, J. H., FUTTERMAN, S., SATOH, K. and MEROLA, L. O. (1963) Factors Affecting the Formation of Sugar Alcohols in the Ocular Lens. Biochim. Biophys. Acta. 74, 340-350.

* KAHN, H.A., LIEBOWITZ, H.M., GANLEY, J.P., KINI, M.M., COLTON, T., NICKERSON, R.S. and DAWBER, T.R. (1977a) The Framingham Eye Study. I. Outline and Major Prevalence Findings. Amer. J. Epidem. 106, 17-32.

** KAHN, H.A., LIEBOWITZ, H.M., GANLEY, J.P., KINI, M.M., COLTON, T., NICKERSON, R.S. and DAWBER, T.R. (1977b) The Framingham Eye Study. II. Association of Ophthalmic Pathology with Single Variables Previously Measured in the Framingham Heart Study. Amer. J. Epidem 106, 33-41.

KINOSHITA, J. H. and MEROLA, L. O. (1958) The Distribution of Glutathione and Protein Sulphydryl Groups in Calf and Cattle Lenses. Am. J. Ophthal. 46, 38-42.

KINOSHITA, J. H. and MEROLA, L. O. (1973) Oxidation of Thiol Groups of the Human Lens. In, The Human Lens in Relation to Cataract. Ciba Foundation Symposium 19 (New Series) pp.173-180.

KINOSITA, J. H., MEROLA, L. O. and DIKMAK, E. (1962a) Osmotic Changes in Experimental Galactose Cataracts. Exp. Eye Res. 1, 405-410.

KINOSHITA, J. H., MEROLA, L. O., SATOH, K. and DIKMAK, E. (1962b) Osmotic Changes Caused by the Accumulation of Dulcitol in the Lenses of Rats Fed on Galactose. Nature 194, 1085-1087.

KIRBY, D. B. (1933) Cataract and Diabetes. A.M.A. Arch. Ophthal. 9, 966-973.

KIRBY, T. S., ACHOR, R. W. P., PERRY, H. O. and WINKLEMAN, R. K. (1962) Cataract Formation After Triparanol Therapy. Arch. Ophthalmol. 68, 486-489.

KIRBY, D. B. and WEINER, R. V. E. (1933) Carbohydrate Tolerance in Elderly Patients with Cataract. Arch. Ophthalmol. 10, 25-27.

KIRWAN, E. O'G. (1933) Diabetic Cataract. Brit. J. Ophthalmol. 17, 346-351.

KOCH, H-R (1976) Klinische und Experimentalle Untersuchungen uber den Einflus von Corticosteroiden auf die Augenlinse. Habilitationsschrift, Bonn.

KOCH, H-R., DOLDI, K. and HOCKWIN, O. (1976) Naphthalene Cataracts in Rats. Association of Eye Pigmentation and Cataract Development. Docum. Ophthal. 8, 293-303.

KOCH, H-R., HOCKWIN, O. and OHRLOFF, C. (1976) Metabolic Disorders of the Lens. Metabol. Ophthalmol. 1, 55-62.

KOCH, H-R., KREMER, F., HOCKWIN, O. and KAUFMANN, H. (1977) Formation of Radiation Cataracts in Rats After Monolateral Carotid Ligature. Biochemical Results. Ophthal. Res. 9, 31-37.

KRAMPS, H. A., HOENDERS, H. J. and WOLLENSAK, J. (1976) Protein Changes in the Human Lens During Development of Senile Nuclear Cataract. Biochim. Biophys. Acta. 434, 32-43.

KRAUSE, A. D. (1932) Chemistry of the Lens.I.Composition of Alluminoid and Alpha-Crystallin. Arch. Ophthal. 8, 166-172.

KRAUSE, A. D. (1933) Chemistry of the Lens. II. Composition of Beta Crystallin, Albumin (Gamma Crystallin) and Capsule. Arch. Ophthal. 9, 617-624.

KURZEL, R. B., WOLHARSHT, M., YAMANASHI, B. S., STATON, G. W. and BORKMAN, R. F. (1973) Tryptophan Excited States and Cataracts in the Human Lens. Nature 241, 132-133.

LASSER, A. and BALAZS, E. A. (1972) Biochemical and Fine Structure Studies on the Water-Insoluble Components of the Calf Lens. Exp. Eye Res. 17, 65-71.

LAWRENCE, R. D. (1946) Temporary Cataracts in Diabetes. Brit. J. Ophthal. 30, 78-81.

LERMAN, S. (1972) Lens Proteins in Aging and Cataract Formation. In Contemporary Ophthalmology, (Ed. Bellows, J. G.) pp.476-493. Williams and Wilkins, Baltimore.

LERMAN, S. (1976) Lens Fluorescence in Aging and Cataract Formation. Documenta. Ophthal. 8, 241-260.

LERMAN, S. and BORKMAN, R. (1976) Spectroscopic Evaluation and Classification of the Normal, Ageing and Cataractous Lens. Ophthal. Res. 8, 335-353.

LERMAN, S., ZIGMAN, S. and FORBES, W. F. (1968) Insoluble Protein Fraction of the Lens. Exp. Eye Res. 7, 444-448.

LI, L-K. (1974) Physical and Chemical Changes in Alpha-crystallin During Maturation of Lens Fibres. Exp. Eye Res. 18, 383-393.

LIEM-THE, K. N. and HOENDERS, H. J. (1974a) Characterisation of the Soluble Proteins from Rabbit Eye Lens. Exp. Eye Res. 18, 143-152.

LIEM-THE, K. N. and HOENDERS, H. J. (1974b) HM-crystallin as an Intermediate in the Conversion of Water-soluble into Water-insoluble Rabbit Lens Proteins. Exp. Eye Res. 19, 549-558.

LIEM-THE, K. N., RINGENS, P. J. HOLTSLAG, J. C. W. M. and HOENDERS, H. J. (1978) Changes in the Proportion and Composition of Structural Proteins During the Development of X-ray Cataract in Rabbit Lens. Interdiscipl. Topics Geront. 12, 105-118.

LIEM-THE, K. N., STOLS, A. L. H. and HOENDERS, H. J. (1975) Further Characterisation of HM-crystallin in Rabbit Lens. Exp. Eye Res. 20, 307-316.

LIEM-THE, K. N., STOLS, A. L. H., JAP, P. H. K. and HOENDERS, H. J. (1975) X-ray induced Cataract in Rabbit Lens. Exp. Eye Res. 20, 317-328.

MacAVOY, J. W. and VAN HEYNINGEN, R. (1976) Changes in the Cells of the Lens Bow and Epithelium of Tryptophan Deficient Rats. Les Colloques de l'INSERM. 245-250.

MACH, H. (1963) Untersuchungen von Linseneiweiss und Mikroelektrophorese von wasserloslichen Eiwess im Altersstar. Klin. Mbl. Augenheilk 143, 689-710.

MAJOR, R. H. and CURRAN, E. J. (1925) Diabetic Cataracts in an Infant J.A.M.A. 84, 674

MALINOWSKI, K. and MANSKI, W. (1980) An Immunochemical Study of the Different Proteins in the Beta and Gamma Crystallin Families. Exp. Eye Res. 30, 519-526.

MALINOWSKI, K. and MANSKI, W. (1980) Immunochemical Studies on Lens Protein-Protein Complexes. II. On the Mechanism of Alpha-Beta-Gamma Crystallin Complex Formation. Exp. Eye Res. 30, 527-536.

MALINOWSKI, K. and MANSKI, W. (1980) Immunochemical Studies on Lens Protein-Protein Complexes. III. Age-Related Changes in the Stability of Lens Alpha Crystallin Containing Complexes. Exp. Eye Res. 30, 537-543.

MANN, I. (1966) Culture, Race, Climate and Eye Disease : an Introduction to the Study of Geographical Ophthalmology. C.C. Thomas, Springfield, Ill.

MANSKI, W., BEHRENS, M. and MARTINEZ, C. (1968) Immunochemical Studies on Albuminoid. Exp. Eye Res. 7, 164-171.

MANSKI, W., MALINOWSKI, K and BONITSIS, G. (1980) Immunochemical Studies on Lens Protein-Protein Complexes. I. The Heterogeneity and Structure of Complexed Alpha Crystallin. Exp. Eye Res. 29, 625-635.

MANSKI, W., and MARTINEZ, C. (1971) Immunochemical Studies on Albuminoid. II. Changes Associated with Age. Exp. Eye Res. 12, 206-211.

MARAINI, G. and MANGILI, R. (1973a) Characteristics of Alpha-crystallin in human Senile Cataract. Exp. Eye Res. 16, 123-129.

MARAINI, G. and MANGILI, R. (1973b) Differences in Proteins and in the Water Balance of the Lens in Nuclear and Cortical Types of Cataract. Human Lens in Relation to Cataract. Ciba Foundation Symposium 19 pp. 79-95.

MARAINI, G. and PESCATORI, A. (1972) Changes in Weight and in Protein: Water Ratio of the Lens in Human Senile Cataracts. Ophthal. Res. 3, 108-113.

McGUINNESS, R. (1967) Association of Diabetes and Cataract.Brit. Med. J. 11, 416-418.

MEHTA, P. D. and LERMAN, S. (1970) Immunochemical Relationship Between Soluble and Insoluble Lens Proteins. Ophthal. Res. 1, 10-20.

MEHTA, P. D. and MAISEL, H. (1968) Changes in Human Lens Weight and Protein with Age. Biochemistry of the Eye. Symp. Tutzing Castle, 1966. Karger, Basel/New York. pp.271-276.

MICHON, J. and KINOSHITA, J. H. (1968a) Experimental Miotic Cataract I. Effects of Miotics on Lens Structure, Cation Content and Hydration. Arch. Ophthalmol. 79, 79-86.

MICHON, J. and KINOSHITA, J. H. (1968b) Experimental Miotic Cataract II. Permeability, Cation Transport and Intermediary Metabolism. Arch. Ophthal. 79, 611-616.

MILLER, A. J., FUJINO, T. and NUFZIGER, M. D. (1967) Lens Findings in Atomic Bomb Survivors. Arch. Ophthal. 78, 697-704.

MILLER, D. (1958) A Case of Anorexia Nervosa in a Young Woman with the Development of Subcapsular Cataracts. Trans. Ophthal. Soc. U.K. 78, 217-222

MIZUNO, G., ELLISON, E., CHIPAULT, J. R. and HARRIS, J. E. (1974) Lipids of the Triparanol Cataract in the Rat. Ophthal. Res. 6, 206-215.

MORNER, C. T. (1894) Untersuchung der Proteinsubstanzen in den Leichtbrechenden Medien des Auges. Z. Physiol. Chem. 18, 61-106.

NIE, N. H., HULL, C. H., JENKINS, J. C., STEINBRENNER, K. and BENT, D. H. (1975) Statistical Package for the Social Sciences. 3rd Edition. McGraw-Hill, New York.

NORDMANN, J. (1949) Le durcissement du crystallin. Ophthalmologica 118, 369-377.

NORDMANN, J. (1972) Problems in Cataract Research. Ophthal. Res. 3, 323-359.

O'BRIEN, C. S. and ALLEN, J. H. (1942) Ocular changes in Young Diabetic Patients. J.A.M.A. 120, 190-192.

O'BRIEN, C. S., MOLSBERRY, J. M. and ALLEN, J. H. (1934) Diabetic Cataract. Incidence and morphology in 126 young diabetic patients. J.A.M.A. 103, 892-897.

OGLESBY, R. B., BLACK, R. L., VON SALLMAN, L. and BUNIM, J. J. (1961)a Cataracts in Rheumatoid Arthritis Patients Treated with Corticosteroids. Description and Differential Diagnosis. Arch. Ophthal. 66, 519-523.

OGLESBY, R. B., BLACK, R. L., VON SALLMANN, L. and BUNIM, J. J. (1961)b Cataracts in Patients with Rheumatic Diseases Treated with Corticosteroids. Arch. Ophthal. 66, 625-630.

OHRLOFF, C., STAFFEL, C., KOCH H-R., WEFERS, V., BOURS, J and HOCKWIN, O. (1978) Experimental Cataracts in Rats Due to Tryptophan-free Diet. Albrecht V. Graefes Arch. Klin. Exp. Ophthal. 205, 73-79.

ONO, S., HIRANO, H. and OBARA, K. (1972a) Presence of Cortisol-binding Proteins in the Lens. Ophthal. Res. 3, 233-240.

ONO, S., HIRANO, H. and OBARA, K. (1972b) Study on the Conjugation of Cortisol in the Lens. Ophthal. Res. 3, 307-310.

ONO, S., HIRANO, H. and OBARA, K. (1972c) Studies on the Inactivation of Cortisol-4-(^{14}C) in the Lenses of Liver-impaired Rats. Ophthal. Res. 4, 160-163.

ONO, S., HIRANO, H. and OBARA, K. (1973) Biochemical Studies on the Pathogenesis of Steroid Cataracts with Particular Reference to the Pituitary-Adrenal-Liver Axis. Exp. Eye Res. 15, 61-68.

OSUNTOKUN, O. and OLVRIN, O. (1973) Cataract and Cataract Extraction in Nigerians. An Evaluation of 567 Extractions. Brit. J. Ophthal. 57, 27-33.

PALMER, W. G. and PAPACONSTANTINOU, J. (1969) Ageing of α-crystallin During Development of the Lens. Proc. Natl. Acad. Sci. 64, 404-410.

PAPACONSTANTINOU, J. (1965) Biochemistry of Bovine Lens Proteins. II. The γ-crystallins of Adult Bovine, Calf and Embryonic Lenses. Biochim. Biophys. Acta. 107, 81-90.

PATTERSON, J. W. (1951) Course of Diabetes and Development of Cataract After Injecting Dehydroascorbic Acid and Related Substances. Am. J. Physiol. 165, 61-65.

PATTERSON, J. W. (1952) Development of Diabetic Cataracts. Am. J. Ophthal. 35, 68-72.

PHILIPSON, B. (1969a) Distribution of Protein Within the Normal Rat Lens. Invest. Ophthal. 8, 258-270.

PHILIPSON, B. (1969b) Distribution of Protein Within Lenses with X-ray Cataract. Invest. Ophthal. 8, 271-280.

PHILIPSON, B. (1969c) Galactose Cataract: Changes in Protein Concentration During Development. Invest. Ophthal. 8, 281-289.

PHILIPSON, B. T. (1973) Changes in the Lens Related to the Reduction in Transparency. Exp. Eye Res. 16, 29-39.

PHILIPSON, B. T. and FAGERHOLM, P. P. (1973). Lens Changes Responsible for Increased Light Scattering in some Types of Senile Cataract. In The Human Lens in Relation to Cataract. pp.45-58. Ciba Foundation Symposium.

PHILIPSON, B., KAUFMAN, P. L., FAGERHOLM, P., AXELSSON, U. and BARANY, E. H. (1979) Echothiophate Cataracts in Monkeys. Arch. Ophthalmol. 97, 340-346.

PIATIGORSKY, J., KADOR, P. F. and KINOSHITA, J. N. (1980) Differential Synthesis and Degradation of Protein in the Hereditary Philly Mouse Cataract. Exp. Eye Res. 30, 69-78.

PIRIE, A. (1968) Colour and Solubility of the Proteins of Human Cataracts. Invest. Ophthal. 7, 634-650.

PIRIE, A. (1971) Formation of N'-formylkyneurenine in Proteins from Lens and other Sources by Exposure to Sunlight. Biochem. J. 125, 203-208.

PIRIE, A. (1972) The Effect of Sunlight on Proteins of the Lens. In Contemporary Ophthalmology, Ed. J. G. Bellows, Williams and Wilkins, Baltimore.

PIRIE, A. and VAN HEYNINGEN, R. (1964) The Effect of Diabetes on the Content of Sorbitol, Glucose, Fructose and Inositol in the Human Lens. Exp. Eye Res. 3, 124-131.

***

POULARD, A. (1923) Traité d'ophthalmologie, Paris, Masson and Cie. pp.881-1064.

RATHBUN, W. B., HARRIS, J. E., VAGSTAD, C. and GRUBER, L. (1973) The Reversal of Triparanol-induced Cataracts in the Rat. IV. Reduced Sulphydryl Groups in Soluble Protein and Glutathione. Invest. Ophthalmol. 12, 388-390.

RATHBUN, W. B., HOUGH, M., GRUBER, L. and HARRIS, J. E. (1978) The Reversal of Triparanol-induced Cataract in the Rat. V. Activity Levels of ATPase and Three Enzymes of Glutathione Metabolism. Interdiscipl. Topics Geront. 12, 132-140.

REES, J. R. and PIRIE, A. (1967) Possible Reactions of 1,2-Naphthaquinone in the Eye. Biochem. J. 102, 853-863.

*** see page 171

RESNIK, R. A. (1957) Alpha Crystallin of Calf Lens. Am. J. Ophthalmol. 44, 357-362.

RICKETTS, H. T., PETERSON, E. S., LINTS, H., TUPIKOVA, N. and STEINER, P. E. (1959) Degenerative Lesions in Dogs with Experimental Diabetes. Diabetes 8, 298-306.

RIDLEY, N.C. (1920) Clin. J. London 49, 41-44.

RINGENS, P. J. LIEM-THE, K. N., HOENDERS, H. J. and WOLLENSAK, J. (1978) Normal and Cataractous Human Lens Crystallins. Interdiscipl. Topics Geront. 12, 193-211.

***

ROGERS, K. M. and AUGUSTEYN, R. C. (1978) Gluthathione Reductase in Normal and Cataractous Human Lenses. Exp. Eye Res. 27, 719-721.

ROMER, P. (1912) Zur Pathogenese der Katarakt. Klin. Manstbl. f. Augenh. 50, 268.

ROY, D. and SPECTOR, A. (1976a) Absence of Low-molecular Weight Alpha-crystallin in Nuclear Region of Old Human Lenses. Proc. Natl. Acad. Sci. 73, 3484-3487.

ROY, D. and SPECTOR, A. (1976b) High Molecular Weight Protein from Human Lenses. Exp. Eye Res. 22, 273-279.

ROY, D. and SPECTOR, A. (1978a) Human Insoluble Lens Proteins. I. Separation and Partial Characterisation of Polypeptides. Exp. Eye Res. 26, 429-444.

*** see page 171

ROY, D. and SPECTOR, A. (1978b) Human Insoluble Lens Protein. II Isolation and Characterisation of a 9600 Dalton Polypeptide. Exp. Eye Res. 26, 445-460.

RUTTENBURG, G. (1965) The Insoluble Proteins of Bovine Crystallin Lens. Exp. Eye Res. 4, 18-23.

SAID, F. S. and WEALE, R. A. (1959) The Variation with Age of the Spectral Transmissivity of the Living Human Crystalline Lens. Gerontologia. 3, 213-231.

SATOH, K. (1973) Fluorescence in Human Lens. Exp. Eye Res. 16, 167-172.

SCHACHER, R. A. and SOLIN, S. A. (1975) The Microscopic Protein Structure of the Lens with a Theory for Cataract Formation as Determined by Raman Spectroscopy of Intact Bovine Lenses. Invest. Ophthalmol. 14, 380-396.

SCHOENMAKERS, J. G. G. and BLOEMENDAL, H. (1968a) Subunits of Alpha-crystallin from Adult and Embryonic Cattle Lens. Nature 220, 790-791.

SCHOENMAKERS, J. G. G. and BLOEMENDAL, H. (1968b) Non identical Subunits in α-crystallin. Biochem. Biophys. Res. Comm. 31, 257-261.

SHEPHERDSON, H. C. and CRAWFORD, J. W. (1931) Ocular Findings in Diabetes: Comment on an Unselected Group of Adult Diabetics. California and West Med. 35, 111-117.

SIEZEN, R. J. and HOENDERS, H. J. (1978) Architecture of α-crystallin: Surface Probing by Limited Proteolysis. Interdiscipl. Topics Geront. 12, 221-231.

SIEZEN, R. J., BINDELS, J. G. and HOENDERS, H. J. (1979) The Inter-relationship Between Monomeric, Oligomeric and Polymeric α-crystallin in the Calf Lens Nucleus. Exp. Eye Res. 28, 551-567.

SLINGSBY, C. and CROFT, L. R. (1972) Microheterogeneity at the C-Terminus of γ-crystallin Fraction IVb. Biochem. J. 127, 609-610.

SORSBY, A. (1951) Genetics in Ophthalmology. Butterworths, London.

SORSBY, A. (1962) Cataract: Some Statistical and Genetic Aspects. Exp. Eye Res. 1, 296-299.

**

SPECTOR, A., FREUND, T., LI, L-K., and AUGUSTEYN, R. C. (1971) Age-dependent Changes in the Structure of Alpha-crystallin. Invest. Ophthal. 10, 677-686.

SPECTOR, A. and KATZ, E. (1965) The Deaggregation of Bovine Lens α-crystallin. J. Biol. Chem. 240, 1979-1985.

** SPECTOR, A. (1974) Report on the National Eye Institute Cataract Workshop. Invest. Ophthal. 13 325-332.

SPECTOR, A., LI, L-K., AUGUSTEYN, R. C., SCHNEIDER, A. and FREUND, T. (1971) α-crystallin. The Isolation and Characterisation of Distinct Macromolecular Fractions. Biochem. J. 124, 337-343.

SPECTOR, A., LI, S. and SIGELMAN, J. (1974) Age Dependent Changes in the Molecular Size of Human Lens Proteins and Their Relationships to Light Scatter. Invest. Ophthal. 13, 795-798.

SPENCER, R. W. and ANDELMAN, Y. S. (1965) Steroid Cataracts. Posterior Subcapsular Cataract Formation in Rheumatoid Arthritis Patients on Long-Term Steroid Therapy. Arch. Ophthal. 74, 38-41.

STAUFFER, J., ROTHSCHILD, C., WANDEL, T. and SPECTOR, A. (1974) Transformation of α-crystallin Polypeptide Chains with Ageing. Invest. Ophthal. 13, 135-146.

STREETEN, B. W. and ESLAGHIAN, J. (1978) Human Posterior Subcapsular Cataract. Arch. Ophthalmol. 96, 1653-1658.

TAPASZTO, I. (1962) Changes in the Proteins of the Human Eye Lens During the Development of the Cataract. Acta Ophthal. 40, 77-84.

TAYLOR, H. R. (1980) The Environment and the Lens. Brit. J. Ophthal. 64, 303-310.

TESTA, M., ARMAND, G. and BALAZS, E. A. (1965) Separation of the Soluble Proteins of Bovine Lenses on Polyacrylamide Gels. Exp. Eye Res. 4, 327-339.

TESTA, M., FIORE, C., BOCCI, N. and CALABRO, S. (1968) Effect of Oxidation on Sulphydryl Groups in Lens Proteins. Exp. Eye Res. 7, 276-290.

THOMSON, I., WILKINSON, C. E., BURNS, A. T. H., TRUMAN, D. E. S. and CLAYTON, R. M. (1978) Characterisation of Chick Lens Soluble Proteins and the Control of Their Synthesis. Exp. Eye Res. 26, 351-362.

TROKEL, S. (1962) The Physical Basis for Transparency of the Crystalline Lens. Invest. Ophthal. 1, 493-501.

TRUMAN, D. E. S., BROWN, A. G. and RAO, K. V. (1971) Estimation of the Molecular Weights of Chick β- and γ-crystallins and their Subunits by Gel Filtration. Exp. Eye Res. 12, 304-310.

TRUMAN, D. E. S. and CLAYTON, R. M. (1974) The Subunit Structure of Chick β-crystallin. Exp. Eye Res. 18, 485-494.

TRUSCOTT, R. J. W. and AUGUSTEYN, R. C. (1977a) Changes in Human Lens Proteins During Nuclear Cataract Formation. Exp. Eye Res. 24, 159-170.

TRUSCOTT, R. J. W. and AUGUSTEYN, R. C. (1977b) Oxidative Changes in Human Lens Protein During Senile Nuclear Cataract Formation. Biochim. Biophys. Acta. 492, 43-52.

TRUSCOTT, R. J. W. and AUGUSTEYN, R. C. (1977c) The State of Sulphydryl Groups in Normal and Cataractous Human Lenses. Exp. Eye Res. 25, 1-10.

van DAM, A. F. (1966) Purification and Composition Studies of β_s-crystallin. Exp. Eye Res. 5, 255-266.

van HAARD, P. M. M. (1980) Oxidative Challenge to Aging Human Lens Leading to Nuclear Colour. Ph.D. Thesis, University of Nijmegen.

van HAARD, P. M. M., KRAMPS, J. A., HOENDERS, H. J. and WOLLENSAK, J. (1978) Development of Non-Disulphide Covalent Cross-Links in Nuclear Cataractogenesis. Interdiscipl. Topics Geront. 13, 212-224.

van HEYNINGEN, R. (1959) Formation of Polyols by the Lens of the Rat with "Sugar" Cataracts. Nature 184, 194-195.

van HEYNINGEN, R. (1962) The Sorbitol Pathway in the Lens. Exp. Eye Res. 1, 396-404.

van HEYNINGEN, R. (1976) Experimental Studies on Cataract. Invest. Ophthalmol. 15, 685-697.

van HEYNINGEN, R. (1979) Napthalene Cataracts in Rats and Rabbits: A Resume. Exp. Eye Res. 28, 435-439.

van HEYNINGEN, R. and PIRIE, A. (1967) The Metabolism of Naphthalene and its Toxic Effect on the Eye. Biochem J. 102, 842-852.

van KAMP, G. J. and HOENDERS, H. J. (1973) The Distribution of the Soluble Proteins in the Calf Lens. Exp. Eye Res. 17, 417-426.

van KLEEF, F. S. M. (1974) Post-Synthetic Modifications of Bovine α-Crystallin. Ph.D. Thesis, University of Nijmegen.

van KLEEF, F. S. M., NIJZINK, M. J. C. M. and HOENDERS, H. J. (1974) Nine polypeptide Chains in α-crystallin. Exp. Eye Res. 18, 201-204.

van KLEEF, F. S. M., WILLEMS-THIJSSEN, W. and HOENDERS, H. J. (1976) Intracellular Degredation and Deamidation of α-Crystallin Subunits. Eur. J. Biochem. 66, 447-483.

VARMA, S. D. and KINOSHITA, J. H. (1976) Inhibition of Lens Aldose Reductase by Flavonoids; Their Possible Role in the Prevention of Diabetic Cataracts. Biochem. Pharmacol. 25, 2505-2513.

VERMORKEN, A. J. M., HERBRINK, P. and BLOEMENDAL, H. (1977). Synethesis of Lens Protein in vitro : Formation Beta Crystallin. Eur. J. Biochem. 78, 617-622.

VOGT, A. (1931) Lehrbuch und Atlas de Spaltlamperimikroskopic des Lebenden Auges. 2. Teil: Linse und Zonula. Springer, Berlin.

von SALLMAN, L., REID, M. E., GRIMES, P. and COLLINS, L. M. (1959) Tryptophan Deficiency Cataract in Guinea Pigs. Arch. Ophthalmol. 62, 662-672.

von SALLMAN, L., GRIMES, P. and COLLINS, E. (1963) Triparanol Induced Cataract in Rats. Arch. Ophthalmol. 70, 522-530.

WAGNER, B. J. and FU, S-C. J. (1978) Characterisation of Lens Proteins II. γ-Crystallin of Normal and Cataractous Rat Lenses. Exp. Eye Res. 26, 255-265.

WALEY, S. G. (1965) The Problem of Albuminoid. Exp. Eye Res. 4, 293-297.

WALEY, S. G. (1969) Nomenclature for the Polypeptide Chains of α-crystallin. Exp. Eye Res. 8, 477-478.

WEALE, R. A. (1979) Sex, Age and the Birefringence of the Human Crystalline Lens. Exp. Eye Res. 29, 449-461.

WILLIAMSON, J. and DALAKOS, T. G. (1967) Posterior Subcapsular Cataracts and Macular Lesions After Long-Term Corticotrophin Therapy. Br. J. Ophthalmol. 51, 839-842.

WILLIAMSON, J., PATERSON, R. W. W., McGAVIN, D. D. M., JASUNI, M. K., BOYLE, J. A. and DOIG, W. M. (1969) Posterior Subcapsular Cataracts and Glaucoma Associated with Long-Term Oral Corticosteroid Therapy. Brit. J. Ophthal. 53, 361-372.

WRIGLEY, C. (1968) Gel Electrofocusing. A Technique for Analysing Multiple Protein Samples by Isoelectric Focusing. Science Tools 15, 17-23.

YAMASHI, B. S. and ZUCLICH, J. A. (1978) Triplet States of Tryptophan, N-formylkynurenine and U. V. Irradiated Lens Proteins. Ophthal. Res. 10, 140-149.

YIM, S. H. (1978) Discriminant Analysis of the Soluble Protein Composition of Individual Cataractous Human Lenses. M.Sc. Thesis, University of Edinburgh.

YORK, A. T., HAMADA, S. and MANSKI, W. (1971) Immunochemical Studies on Crystallins and Albuminoids in Galactose Cataracts of Inbred Rats. Ophthal. Res. 2, 273-284.

YORK, A. T., MARTINEZ, C. and MANSKI, W. (1972) Immunochemical Studies on Crystallins and Albuminoids in Human Senile Cataract. Ophthal. Res. 3, 183-191.

ZIGLER, J. S. (1978) Age-related Changes in the Polypeptide Composition of β-crystallin from Bovine Lens. Exp. Eye Res. 26, 537-546.

ZIGLER, J. S. and SIDBURY, J. B. (1973) Structure of Calf-lens β-crystallin. Exp. Eye Res. 16, 207-214.

ZIGLER, J. S. and SIDBURY, J. B. (1977) Studies on Lens Proteins from the Smooth Dogfish. Evidence for Evolutionary Conservatism in Vertebrate β-crystallin. Ophthal. Res. 9, 92-98.

ZIGLER, J. S. SIDBURY, J. B., YAMANASHI, B. S. and WOLBARSHT, M. (1976) Studies of Brunescent Cataracts. I. Analysis of Free and Protein Bound Amino Acids. Ophthal. Res. 8, 379-387.

ZIGMAN, S. (1971) Eye Lens Colour: Formation and Function. Science 171, 807-809.

ZIGMAN, S., GROFF, J., YULO, G. and GREISS, G. (1976) Light Extinction and Proteins in the Lens. Exp. Eye Res. 23, 555-562.

ZIGMAN, S., SCHULTZ, J. and YULO, T. (1969) Chemistry of Lens Nuclear Sclerosis. Biochem. and Biophys. Res. Commun. 35, 931-938.

ZIGMAN, S., SCHULTZ, J. and YULO, T. (1970) Isoelectric Focusing of γ-crystallins. I. Characterisation of Rat Lens Components. Biochim. Biophys. Acta. 221, 576-582.

ZIGMAN, S., SCHULTZ, J., YULO, T. and GREISS, G. (1971a) Fed. Proc. Fed. Amer. Soc. Exp. Biol. 30

ZIGMAN, S., SCHULTZ, J. and YULO, T. (1971b) Isoelectric-focusing of γ-crystallins. II. Ageing Variations in the Rat Lens. Ophthal. Res. 2, 110-115.

ZWAAN, J. (1968) Electrophoretic Studies on the Heterogeneity of Chicken Lens Crystallins. Exp. Eye Res. 7, 461-472.

BORNSTEIN, J. and NELSON, J. F. (1949) Observation on the Effect of High-fat Diet in Alloxan Diabetic Rats. M. J. Australia 36, 121-126.

CHARALAMPOUS, F. C. and HEGSTED, D. M. (1950) Effect of Age and Diet on Development of Cataracts in the Diabetic Rat. Am. J. Physiol. 161, 540-544.

CREIGHTON, M. O. and TREVITHICK, J. R. (1979) Cortical Cataract Formation Prevented by Vitamin E and Glutathione. Exp. Eye Res. 29, 689-693.

DUNCAN, G. and van HEYNINGEN, R. (1976) Differences in the Calcium Binding Capacity of Normal and Cataractous Lenses. Docum. Ophthal. Proc. Ser. 8, 229-232.

HAMMAR, H. (1965) The Influence of Different Fat Diets on the Fluorescence of the Eye Lens and of Serum in Allexan Diabetic Rats. Acta Ophthal. 43, 61-74.

HANNO, H. A. and WEISS, D. I. (1961) Hypoparathygroidism, Pseudo-hypoparanthyroidism and Pseudo-Pseudohypoparanthyroidism. Arch. Ophthal. 65, 238-242.

HEFFLEY, J. D. and WILLIAMS, R. J. (1974) The Nutritional Teamwork Approach. Prevention and Regression of Cataracts in Rats. Proc. Nat. Acad. Sci. 71, 4164-4168.

POHJOLA, S. (1962) Ocular Manifestations of Idiopathic Hypoparathyroidism. Acta Ophthal. 40, 255-264.

RODRIQUES, R. R. and KREHL, W. A. (1951) The Influence of Diet and Insulin on the Incidence of Cataracts in Diabetic Rats. Yale J. Biol. Med. 24, 103-108.

Interdisciplinary Topics in Gerontology
Editor: H.P. von Hahn, Basel
Publishers: S. Karger, Basel
Reprint (Printed in Switzerland)

Interdiscipl. Topics Geront., vol. 13, pp. 183–192 (Karger, Basel 1978)

Analysis of the Crystallin Composition of Individual Human Lenses: Characteristic Modifications Associated with Different Cataracts

J. Cuthbert[1], *Ruth M. Clayton, D.E.S. Truman, C.I. Phillips and R.S. Bartholomew*

Institute of Animal Genetics, and Department of Ophthalmology, University of Edinburgh, Edinburgh

Introduction

Modifications of the protein content of the lens associated with cataract formation have been reported regularly for many years: recent reviews are those of *Nordmann* (1972, 1977), *Barber* (1973) and *Harding and Dilley* (1976). Changes include loss of low molecular weight crystallins, formation of high molecular weight complexes and insolubilisation associated with cross-linking (*Roy and Spector,* 1976; *Kramps et al.,* 1976).

Cataracts are customarily divided into nuclear cataracts, further subdivided by colour; or cortical, which may be described by area (posterior, anterior, peripheral) and further described by the appearance of the lesion (vacuoles, clefts, granules, separated fibres). Since there is an ontogenic shift in the crystallin composition of successively formed lens fibres (*Clayton,* 1974) and marked differences in the quantitative composition of different regions of the lens (*Bours et al.,* 1976) the site and nature of the lesion must be reflected by the changes in the crystallin profile. In addition, membrane properties and growth conditions affecting mitosis and metabolism of lens cells produce modifications in the profiles of crystallin synthesis (*Clayton et al.,* 1976a).

To date analyses of the crystallin content of human cataractous lenses have been based on pooled material, and most studies have used methods of crystallin separation of moderate resolving power. Such studies are designed to determine

[1] *J. Cuthbert* is in receipt of a postgraduate scholarship from the Faculty of Medicine, University of Edinburgh.

major changes in cataractous lenses rather than to search for possible differences amongst varieties of cataract. However, several lines of evidence show that there is a range of different kinds of modification of the crystallin profile. For example, *Dilley and Pirie* (1974) found insolubilisation of proteins of the nucleus, without modification of the cortex, during the development of nuclear cataract and immunoelectrophoretic analyses of X-ray and galactose-induced experimental cataracts show similar but not identical modification of crystallin content (*Manski et al.*, 1976).

Recent studies of the crystallin profile of genetic cataracts in animals have shown distinctive patterns of change. Two different cataract mutants in the mouse produce genotype-specific characteristic distortions of the quantitative profile of crystallin subunits in the water-soluble (WS) and urea-soluble (US) compartments (*Day and Clayton,* 1972; *Truman et al.*, unpublished). Two genetically distinct lens defects in the chick of similar morphopathology are similarly distinguished by their crystallin profile (*Clayton et al.*, 1976a, b).

A cataractous mutant in the axolotl also has several quantitative modifications of the crystallin composition (*Brahma and Bours,* 1976).

An examination of the data presented by *Maraini and Mangili* (1973) suggests that the quantitative balance in α-crystallin subunits differs when normal lenses and nuclear, posterior cortical and anterior cortical cataracts are compared. *Hoenders* (1976) also found differences in the subunit balance of pooled normal and cataractous lenses. There has not yet been an investigation of cataracts of different morphopathology or aetiology to determine whether they may be distinguished by specific changes in crystallin polypeptides, and to determine whether there is significant heterogeneity within classes of cataracts.

Studies of the composition of the soluble crystallins of cataractous lenses might be expected to yield more detailed information if studied by fractionation techniques of high resolution, such as isoelectric focusing (IEF) in non-dissociating conditions (*Bours,* 1971; *Brahma and Bours,* 1976; *Kramps et al.*, 1976). The individual polypeptide chains can be studied by isoelectric focusing in dissociating conditions (*Truman and Clayton,* 1974) and this procedure, which must give even more detailed information than that obtained from analyses of dissociated material, is in principle applicable to both the WS and the US fractions of a cataractous lens.

We present here a study of the changes in the quantitative profiles of WS and US crystallin polypeptide chains in 93 individual cataractous lenses of different types.

Distinctive crystallin profiles have been observed which characterise different types of cataract, suggesting that the location of the lesion and the mechanism of cataractogenesis may be reflected in selective modifications of the quantitative balance of the crystallin subunits.

Materials and Methods

Lenses

Before surgery, each lens was examined by ophthalmoscope and slitlamp microscopy. The classification and detailed appearance of the lens was recorded along with relevant data from the clinical history which was available for each patient. Lenses were frozen on solid CO_2 immediately after extraction and stored at -20 °C (usually for 3 days but for not longer than 2 weeks) until assayed. The wet weight was measured before and the dry weight after homogenisation.

Preparation of the WS and US Fractions

Each lens was homogenised in 0.5 ml 0.01 *M* phosphate buffer, pH 7.2, containing 10 m*M* β-mercaptoethanol (βME), centrifuged for 10 min at 12,000 *g* and the supernatant retained as the WS fraction. The remaining pellet was washed seven times in the buffer to ensure exhaustive extraction of the WS proteins, then resuspended in 0.1 ml 8 *M* urea in 0.1 *M* phosphate buffer plus 10 m*M* βME and left overnight at 4 °C. The suspension was then centrifuged for 30 min at 30,000 *g* and the supernatant retained as the US fraction. The insoluble pellets were retained for assays of the carbohydrate content and for analysis of membrane protein as described in *Clayton et al.* (1976a). These results will be reported elsewhere.

Isoelectric Focusing

The method used is a modification of that described by *Wrigley* (1968). 7.5% polyacrylamide gels, 9.5 cm by 4 mm, incorporating 6 *M* urea and 0.08% of each of the ampholines of ranges pH 3.5–10, pH 4–6 and pH 6–8, were prepared and pre-run at 0.5 mA/gel for 30 min with 50 µl of 3.5% sucrose containing 100 m*M* βME and 2.5% ampholine pH 3.5–10. Samples of the WS fractions of each lens were mixed with an equal volume of dissociating solution (11 *M* urea, 10% sucrose, 100 m*M* βME) and layered on the top of the gels. The gels were run at 0.5 mA/gel for 18 h at 4 °C and then fixed in 12.5% TCA, stained in 0.2% Coomassie brilliant blue R dissolved in a 45% ethanol, 45% H_2O, 10% glacial acetic acid mixture and destained in the same solvent mixture.

Scanning of the Gels

The gels were scanned on a Kipp and Zonen/Skalar KS3 microdensican and traces were obtained on a Bryans XY Recorder 25000 A4, the areas under the peaks being integrated and expressed as a percentage of the total area under the graph (fig. 1). The traces were grouped according to cataract morphology and superimposed by aligning the traces with respect to the individual polypeptides. Where traces were obviously extremely similar in profile a composite standard trace was derived by drawing the curve through the mean values for each peak and trough (fig. 2). The average value for the area under each peak was also calculated (table I, parts I–VI). In order to facilitate identification the traces were divided into six areas and bands numbered within these areas.

Results and Discussion

Our sample of 93 lenses included 51 nuclear cataracts of which 14 were uncomplicated (12 brown and 2 yellow); the remaining 37 having in addition posterior polar or cortical involvement, or both. 48 lenses had cortical defects,

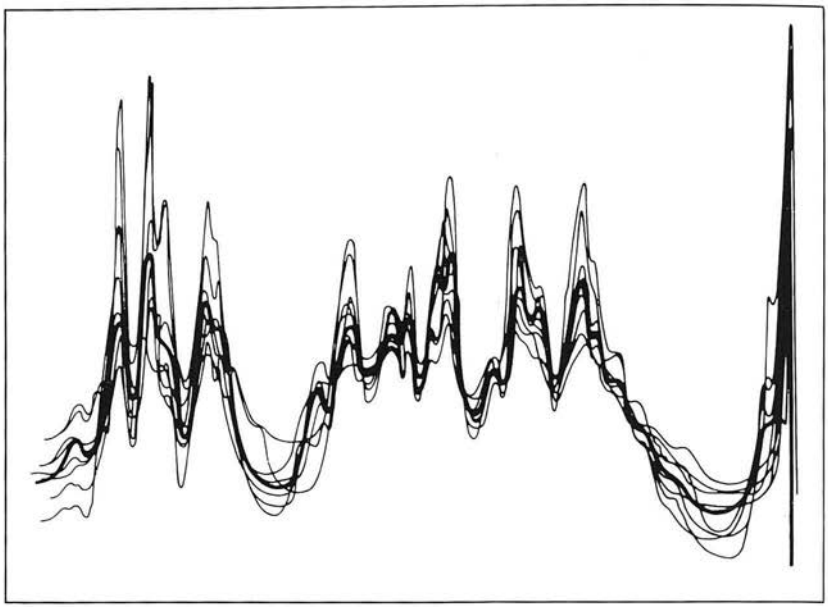


Fig. 1. Superimposition of 12 traces obtained from the WS fraction of 12 individual brown nuclear cataractous lenses and the average trace thus obtained.

only 10 being uncomplicated cortical cataracts, the remainder being combined with posterior polar or nuclear opacities. 7 blue or white dot coronary cataracts were in the sample and 13 lenses came from individuals suffering from specific conditions associated with cataract formation which usually showed appropriate morphopathology, not always uncomplicated. These included 3 cortisone, 4 diabetic and 2 mongol cataracts.

In all cases where a number of individual lenses fell into the same morphopathological classification (for example uncomplicated brown nuclear cataract) the profiles were so closely similar that they could be straightforwardly superimposed, and a representative trace drawn through the mean values. The area under each peak is obtained by integration and the average values for the proportion of the total protein represented in one band allows a more quantitative comparison between corresponding peaks on different traces (table I). Figure 2 shows the standard traces for the WS and US components of a number of groups of cataracts, with each peak identified numerically. There are similarities between the traces of uncomplicated brown and of uncomplicated yellow nuclear cataracts, while the traces from the cortical cataracts are quite different in their profile. Lenses with the same morphopathology but with additional areas of opacity or types of defect deviated from the standard pattern for the

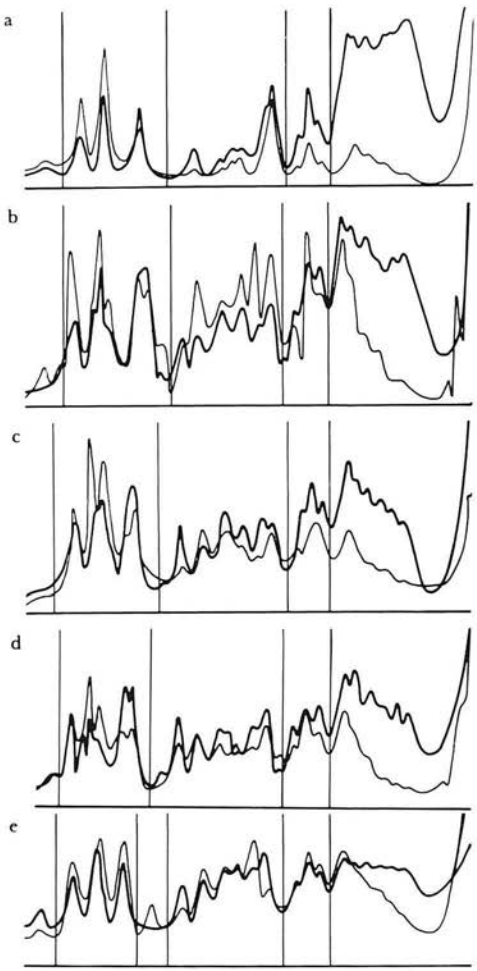


Fig. 2. Average traces of the WS (——) and US (——) fractions of (a) mature white cortical cataract (5 cases); (b) brown nuclear cataract (12 cases); (c) yellow nuclear cataract (2 cases); (d) blue dot plus coronary plus posterior polar subcapsular cataract (4 cases); (e) cataracts extracted from mongol patients (2 cases).

main class in some areas of the trace while retaining certain features characteristic of the entire group: for example; if nuclear and cortical opacities are present, features of both types appear in the trace.

All the cataractous lenses in this survey had a US residue the proportion of which varied but which, in some cataracts (e.g. hypermature cataracts) comprised most of the material. Further analyses of the insoluble fraction of each lens is in progress and will be reported elsewhere.

Table I. Proportions of individual crystallin subunits in the water-soluble and urea-soluble prote fractions from a variety of types of cataractous lenses. Average values for the proportion of ea subunit are calculated from the values obtained for individual gels when scanned and integrated, a are expressed as percentages of the total protein content of either the water-soluble or urea-solub fraction

		I		II			III		
		1	2	1	2	3	0	1	2
Brown	WS	←1.6→		7.6	(7.0, 5.8) 12.8	(4.8, 2.9, 2.1) 9.8		2.4	6.4
Nuclear	US	←1.0→		2.9	4.4	4.8		2.2	2.8
White cortical immature	WS	←2.3→		6.2	(8.1, 6.7) 14.8	(4.2, 5.3) 9.5		4.3	7.0
	US	0.4	1.1	2.2	(2.0, 2.7) 4.7	(2.5, 1.9) 4.4		1.7	2.6
White cortical mature	WS	←2.5→		8.6	22.0	7.6		0.3	1.4
	US	←0.8→		1.2	2.4	1.1		0.7	1.2
White cortical hypermature	WS	←2.3→		16.1	25.5	4.6			
	US	←0.7→		2.3	4.1	2.3		1.0	1.7
Juvenile	WS	5.2	5.9	9.7	10.5	9.4	3.3	2.2	7.3
	US	0.1	0.2	9.3	8.3	(3.2, 2.3) 6.5		0.3	1.8
Cortisone	WS	0.8	1.1	8.6	(6.9, 4.7) 11.6	8.6		3.5	6.4
	US	←0.5→		3.2	4.3	5.3		2.5	2.9
Mongol	WS	←0.2→		7.8	(3.4, 5.6) 9.0	7.2	2.3	2.1	6.0
	US	←1.6→		2.1	4.1	3.9		1.3	2.9

It is evident that some polypeptides are more readily transferred from the WS to the US phase than others. The extent to which this occurs for any subunit is dependent on the nature of the cataract. Such changes may be progressive, as would appear for the comparisons of the cortical cataracts in this series: the percentage of component VI in the US fraction increases steadily as the cataract becomes more mature. However, the fall in the WS and in the US phases of V_{1-6}, suggest that these components may be entering the US phase. Since the total WS

			IV			V						VI (top)	Cases n
4	5	6	1	2	3	1	2	3	4	5	6		
3.4	4.3	6.5	2.5	8.2	3.3	8.6	3.6	2.2	1.2	0.5	0.3	8.5	12
2.8	3.9	3.4	2.9	6.6	3.6	10.5	6.0	6.4	7.4	4.3	9.5	5.5	
4.6	4.5	6.0	2.1	6.2	2.4	6.1	1.8	1.1	0.7	0.2	0.1	16.9	4
2.7	3.6	5.3	2.1	6.4	3.2	10.4	8.0	7.2	6.9	3.8	6.5	13.9	
1.1	←8.0→		0.3	7.4	2.3	6.8	1.1	1.7	2.1	1.4	1.2	22.9	5
1.8	1.5	4.5	1.7	4.8	2.6	10.0	6.8	9.1	8.4	5.0	13.1	21.9	
→				←5.7→					←4.6→			24.9	4
1.6	1.7	5.1	1.2	6.2	1.9	8.3	4.9	5.8	7.7	5.5	13.1	23.0	
5.1	5.9	1.6	←6.7→		2.2	←6.1→				←2.7→		8.0	2
2.4	←11.0→		0.1	11.8	5.0	←20.5→		7.7	5.4	2.3	1.3	3.5	
3.8	2.7	8.1	2.6	7.2	3.5	←11.7→			1.9	1.2	0.2	11.5	3
2.9	2.9	4.1	3.1	6.2	3.4	9.5	5.9	6.1	7.2	4.1	8.4	13.8	
3.8	9.1	2.0	←10.1→		4.9	←11.9→		2.5	←3.1→		1.2	12.5	2
3.0	2.8	6.0	3.0	5.5	3.7	8.6	5.3	6.3	6.3	4.5	8.2	17.3	

fraction has decreased, in hypermature cataracts the apparent increase in the WS fraction of II_2, and VI suggests that these two components are relatively resistant to change. The components in groups III, IV and V become polydisperse in the WS fraction but are still susceptible of resolution in the US fraction: this suggests that the same subunits are less susceptible to post-synthetic modifications when they are in the insoluble phase.

Some regularities were observed in some of the rarer cataracts in the survey. For example, the 3 corticosteroid cataracts showed similarities to each other with the same relative elevation of III_6, and depression of III_5 (fig. 2, table I). 4 lenses were from diabetic patients, 2 had brown nuclear cataracts with other involvement, 1 was a white cortical cataract and 1, a blue dot coronary. 3 of the 4 differed from all other lenses of whatever category, in that III_1, was absent and in 3 there was an increase in the WS components in group V compared to the corresponding type of cataract from a non-diabetic. Thus the percentage of the total soluble crystallins in this group rose from 10.9 to 20.89 is brown nuclear with posterior polar cataract, from 11.26 to 19.5% in cortical cataract and from 14.6 to 23.3% in blue dot coronary cataract. In all these 3 cases the US fraction remained unchanged. However, in the diabetic lens with both nuclear and cortical cataract, there was a marked drop in the WS (from 14.5 to 6.2%) and a rise in the US content of group V (from 30 to 43%) compared to similar non-diabetic lenses. Thus, 2 diabetics showed modifications both of III_1 and of V, WS, and each of the other 2 showed one of these two changes. This apparent existence of subgroups in the diabetic lens patterns may be surprising, but diabetics may be heterogeneous since there are subgroups in the diabetic population with respect to cell surface (histocompatibility) genetics (*Irvine,* 1977).

The lenses with the most strikingly different composition overall were from the 2 juveniles (fig. 2). In addition to the marked changes in overall compositions an extra band between II_3 and III_1 was observed. This band was present in only 2 other lenses of the whole sample, both from mongol patients who were over 50 years old. The traces from the 2 mongol patients are strikingly similar to each other (fig. 2).

We are currently examining a very large number of individual lenses, and present here some data from the first 93 of these. It would appear that this sample is large enough to show that there are several distinctive protein profiles or traces: for both the WS and US phases, and that these characteristic profiles occur in association with particular subgroups of cataracts indicating that the site and nature of the lesion is reflected in a particular quantitative pattern of modifications. On the other hand, our sample is still too small to enable us to analyse the irregularities which in certain specific cases, are superimposed on a standard pattern, thus although unique features were shown by the Coppok cataract and the myotonic cataract only one of each was in the survey. However, the 3 corticosteroid cataracts showed sufficient similarities, as did the 4 lenses

from diabetics, to suggest that continued investigation of these types would be justified. These preliminary findings suggest that previous data based upon pooled material have obscured the possibility that loss or insolubilisation of proteins may not involve all the crystallin subunits to the same extent, nor even the same members of a class of crystallins in each cataractous lens. The modifications that occur may reflect the specific mechanism of cataractogenesis: for example; opacities may follow changes of cell membrane properties but these might affect cell apposition, ion and water transport, or both; or crystallin assembly and packing in the cell might be disturbed by various agencies, such as changes in osmolarity, or oxidative changes: but the effect on the porportions of different subunits remaining extractable in water or urea might well differ in these situations. Our present hope is that discriminatory analyses may provide a link between the various possible causes of cataract and the development of an opacity.

Acknowledgements

We are grateful to the following surgeons for their generous gift of lenses of rare pathology for this survey: *J.S. Cant, J. Hughes, M.J. MacDonald, G.T. Millar, J.W.R. Sarkies and T. Wilson,* to *A. Graham* for conscientious technical assistance and to our colleagues *J.C. Campbell, P. Odeigah* and *D.I. de Pomerai* for helpful discussion.

This work has received support from the W.H. Ross Foundation to Combat Blindness. The work of the lens research group is supported by the MRC and the CRC.

References

Barber, G.W.: Human cataractogenesis. A review. Expl Eye Res. *16:* 85–94 (1973).

Bours, J.: Isoelectric focussing of lens crystallins in thin-layer polyacrylamide gels. A method for detection of soluble proteins in eye lens extracts. J. Chromat. *60:* 225–233 (1971).

Bours, J.; Doepfmer, K., and Hockwin, O.: Isoelectric focussing of crystallins from different parts of the bovine and dog lens in dependence on age. Documenta ophth. *8:* 91–104 (1976).

Brahma, S.K. and Bours, J.: Thin layer isoelectric focussing of soluble and insoluble lens extracts from cataractous and normal Mexican axolotl. *(Ambystoma mexicanum)* Expl Eye Res. *23:* 57–63 (1976).

Clayton, R.M.: Comparative aspects of lens proteins; in *Dousan* The eye, vol 5; pp. 399–494 (Academic Press, London 1974).

Clayton, R.M.; Odeigah, P.G.; De Pomerai, D.I.; Pritchard, D.J.; Thomson, I., and Truman, D.E.S.: Experimental modifications of the quantitative pattern of crystallin synthesis in normal and hyperplastic lens epithelia. Colloques Inserm *60:* 123–136 (1976a).

Clayton, R.M.; Truman, D.E.S.; Hunter, J.; Odeigah, P.G., and De Pomerai, D.I.: Protein synthesis and its regulation in the lenses of normal chicks and in two strains of chicks with hyperplasia of the lens epithelium. Documenta ophth. *8:* 27–37 (1966b).

Day, T.H. and Clayton, R.M.: Multiple changes in lens protein composition associated with the Cat^{Fr} gene in the mouse. Genet. Res. *19:* 241–249 (1972).

Dilley, K.J. and Pirie, A.: Changes to the proteins of the human lens nucleus in cataract. Expl Eye Res. *19:* 59–72 (1974).

Harding, J.J. and Dilley, K.J.: Structural proteins of the mammalian lens: a review with emphasis on changes in development, ageing and cataract. Expl Eye Res. *22:* 1–73 (1976).

Hoenders, H.: Changes in the subunit structure of α-crystallin from bovine and rabbit eye lenses. Documenta ophth. *8:* 105–112 (1976).

Irvine, W.J.: Classification of idiopathic diabetes. Lancet *i:* 638 (1977).

Kramps, H.A.; Hoenders, H.J., and Wollensak, J.: Protein changes in the human lens during development of senile nuclear cataract. Biochim. biophys. Acta *433:* 32–43 (1976).

Manski, W.; York, A.T.; Merrian, G.R.; Worgul, B.V., and Szechter, A.: On the independent action of cataractogenic agents X-rays and galactose. Documenta ophth. *8:* 275–292 (1976).

Maraini, G. and Mangili, R.: Differences in proteins and in the water balance of the lens in nuclear and cortical types of senile cataract; in The human lens in relation to cataract. Ciba Found. Symp. *19:* 79–94 (1973).

Nordmann, J.: Problems in cataract research. Ophthalmic Res. *3:* 323–359 (1972).

Nordmann, J.: Au sujet du vieillissement du cristallin humain et de la cataracte sénile. Adv. Ophthal., vol. 34, pp. 1–73 (Karger, Basel 1977).

Roy, D. and Spector, A.: Human α-crystallin: characterization of the protein isolated from the periphery of cataractous lenses. Biochemistry *15:* 1180–1188 (1976).

Truman, D.E.S. and Clayton, R.M.: The subunit structure of chick β-crystallins. Expl Eye Res. *18:* 485–494 (1974).

Wrigley, C.: Gel electrofocussing, a technique for analysing multiple protein samples by isoelectric focussing. Sci. Tools *15:* 17–23 (1968).

J. Cuthbert, B.Sc., Institute of Animal Genetics, University of Edinburgh, *Edinburgh* (Scotland)

EFFECTS OF ULTRASONICATION OF THE RABBIT LENS IN SITU AS EVALUATED BY ANALYSIS OF CRYSTALLIN COMPOSITION

BY

J. CUTHBERT, C. I. PHILLIPS, R. M. CLAYTON, and D. McG. CLARKSON

Reprinted from

TRANSACTIONS OF THE OPHTHALMOLOGICAL SOCIETIES OF THE UNITED KINGDOM

Vol. 98. Part 4 1978

Trans. ophthal. Soc. U.K. (1978) **98**, 494

Effects of ultrasonication of the rabbit lens *in situ* as evaluated by analysis of crystallin composition

J. CUTHBERT,* C. I. PHILLIPS,† R. M. CLAYTON,* AND D. McG. CLARKSON‡

From the Department of Genetics, and the Department of Ophthalmology,† University of Edinburgh*

Summary

Ultrasonication and aspiration is a recently developed method of lens extraction. We have attempted to determine whether extraction by this procedure is total or partial and, if so, whether certain regions or components are preferentially removed, and also whether the contents of the lens are physico-chemically modified by the process of extraction. The water-soluble and urea-soluble protein fractions from the ultrasonicated lenses and the contralateral intact lenses of six rabbits were analysed by isoelectric focusing. Differences are found in the crystallin content which indicate that extraction is frequently non-representative and that β- and γ-crystallins are modified by the process.

In our study of human cataractous lenses we are comparing the quantitative protein profiles for the water-soluble and urea-soluble fractions in order to ascertain whether there are characteristic modifications which reflect the morphology or aetiology of the different types of cataract (Cuthbert, Clayton, Phillips, Truman, and Bartholemew, 1978 in press). To ensure valid results it is essential that these analyses be wholly comparable. For this reason we have fractionated and analysed the entire lens, since it would not be possible to separate cataractous regions of the lens in any controlled manner.

A recently developed procedure for cataract extraction involves disintegration of the lens by ultrasonic energy and aspiration (Clarkson and Phillips, 1976). An evaluation of the effectiveness of this procedure involves three questions:

(1) Whether there is significant retention of lens fragments or material in the eye, and if so whether this is selective or random;
(2) Whether there is any biochemical or other modification of the lens proteins;
(3) Whether comparative analysis of cataracts may reasonably be made with aspirated sonicated material.

We have therefore compared the protein profiles of rabbit lenses which have been extracted by ultrasonication with those of the contralateral lens which was removed surgically.

Material and methods

The water-soluble and urea-soluble protein fractions were prepared from the following three samples from each rabbit:

(*a*) The intact lens;
(*b*) The aspirate of the lens treated with ultrasound;
(*c*) The residual, non-aspirated fragment from the treated lens.

These fractions were analysed by isoelectric focusing in dissociating conditions (Truman and Clayton, 1974) in order to obtain information about the crystallin content of each.

Ultrasonication and extraction of lenses

In six rabbits, one lens was ultrasonicated and aspirated. The rabbits were anaesthetized and alternately the right or left lens was treated with ultrasonication. The depth of the anterior chamber was maintained by the use of a push-pull machine (Phillips and Wang, 1971; Phillips, 1975), which allowed simultaneous aspiration and irrigation at equal rates. The ultrasonic lens fragmentor was driven at a frequency of 35 KHz by an electrical generator (Clarkson and Phillips, 1976) which delivered a total power input of 2·5w. This enabled the required amplitudes of tip vibration in the region of 50 microns peak-to-peak to be obtained (Clarkson and Phillips, 1975). Any lens fragment remaining after treatment was removed and kept separate. The contralateral lens of each pair was removed surgically as a whole lens. All three specimens were frozen immediately and kept at −20° C until required.

Preparation of water-soluble and urea-soluble protein fractions

Each lens aspirate was centrifuged at 10,000 r.p.m. for 10 min and the supernatants retained as the water-soluble protein fractions. The lens residues and the other intact lenses were individually homogenized in 0·01 M phosphate buffer containing 10 mM β-mercaptoethanol and the homogenates centrifuged at 10,000 r.p.m. for 10 min. These supernatants in turn were retained as the water-soluble protein fractions. The residual pellets were washed six to eight times in the buffer to ensure exhaustive extraction of the water-soluble proteins. The pellets were then re-homogenized in 6 M urea in the above buffer and left overnight at 4° C before being centrifuged at 16,000 r.p.m. for 30 min. The supernatants were retained as the urea-soluble protein fractions, the pellets being the membrane protein fractions. The water-soluble

Address for reprints: Professor C. I. Phillips, FRCS, Eye Pavilion, Chalmers Street, Edinburgh EH3 9HA

‡ Present address: Respiratory Laboratory, City Hospital, Edinburgh

Presented at a meeting of the Scottish Ophthalmological Club at Edinburgh on March 18, 1978

ɔernatants from the aspirates were concentrated by rafiltration so that the protein concentration was mparable with the other water-soluble fractions.

ɔelectric focusing

in-layer isoelectric focusing was carried out on an KB 2117 Multiphor using a 7·5 per cent acrylamide l incorporating 6 M urea and 0·1 per cent of each npholine of pH ranges 3·5–10, 4–6, and 6–8. Samples ntaining 10 to 20 μg protein were mixed with equivalent lumes of dissociating solution (11 M urea, 10 per cent crose, 100 mM β-mercaptoethanol) and absorbed onto nm squares of Whatman No. 1 filter paper. The paper uares were placed on the gel at the anode and a current 45 mA applied across the gel. The current, which creases during isoelectric focusing, was increased ery 5 min for the first 15 min, and then every 10 min til the increase switch was in the maximum position. his maintained the current at 45 mA for approximately min after which it decreased until, after 1·5 hrs, it as about 20 mA. At this stage the gel was removed, ed in 12·5 per cent trichloroacetic acid for 30 min, ained in 0·2 per cent Coomassie Brilliant Blue R in 45 : 45 : 10 ethanol, water, acetic acid mixture, and e-stained in a 65 : 25 : 10 ethanol, water, acetic acid ixture.

esults

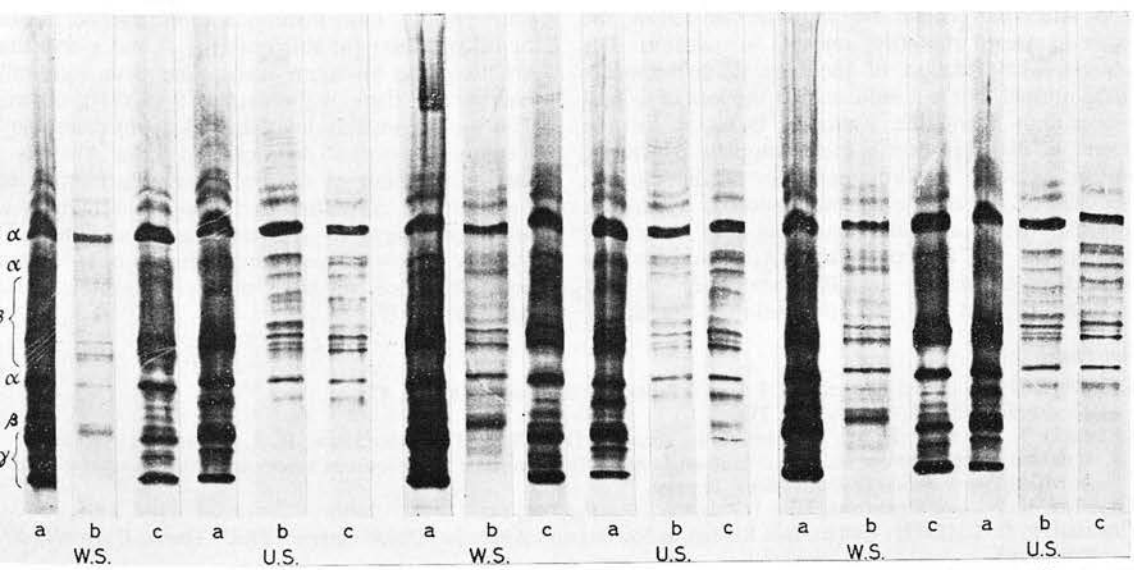
Ve have depended on the work of Liem-The and oenders (1974) for the identification of the bands or this preliminary report. They fractionated ndissociated rabbit crystallins by size on Sepharose B. After immunological identification of these ractions as α-, β-, or γ-crystallins, they were issociated and analysed by isoelectric focusing n the presence of urea.

Sonication of the first three lenses was only about 75 per cent complete. This was calculated by weighing the untreated lens and the residue of the treated lens and estimating the latter value as a percentage of the former. Sonication of the second three lenses was complete in each case, apart from one in which a small fragment remained but from which no crystallins were detectably extracted.

Fig. 1 shows the isoelectric focusing banding patterns of the water-soluble and urea-soluble protein fractions of the whole lens (*a*), the aspirate of the treated lens (*b*), and the non-aspirated residue of the treated lens (*c*) from each of the first three rabbits. In each case, the water-soluble fraction of the non-aspirated residue (*c*), gives a similar banding pattern to that of the intact lens (*a*) in qualitative terms. However, a number of bands, corresponding to the γ-crystallins and some of the β-crystallins as identified by Liem-The and Hoenders, are missing from the patterns obtained from the lens aspirates (*b*). In the case of the urea-soluble protein fractions, neither aspirate nor residue of the sonicated lens gives patterns similar to those of the whole lenses; the bands corresponding to γ- and β-crystallins are reduced in number and a number of extra bands can be seen at the anodal end of the gels.

Only two samples were obtained from each of the second trio of rabbits: the intact lens (*a*), and the aspirate of the completely disintegrated lens (*b*).

Fig. 2 shows the banding patterns obtained from the water-soluble and urea-soluble protein fractions of these samples. The patterns obtained from the water-soluble fractions of the aspirates are different from



G. 1 *Isoelectric focusing in the presence of 6 M urea of the water-soluble (WS) and urea-soluble (US) protein fractions the intact lenses* (a), *the sonicated lens aspirates* (b), *and the residual non-aspirated fragments of the treated lenses* (c) *om the first three rabbits*

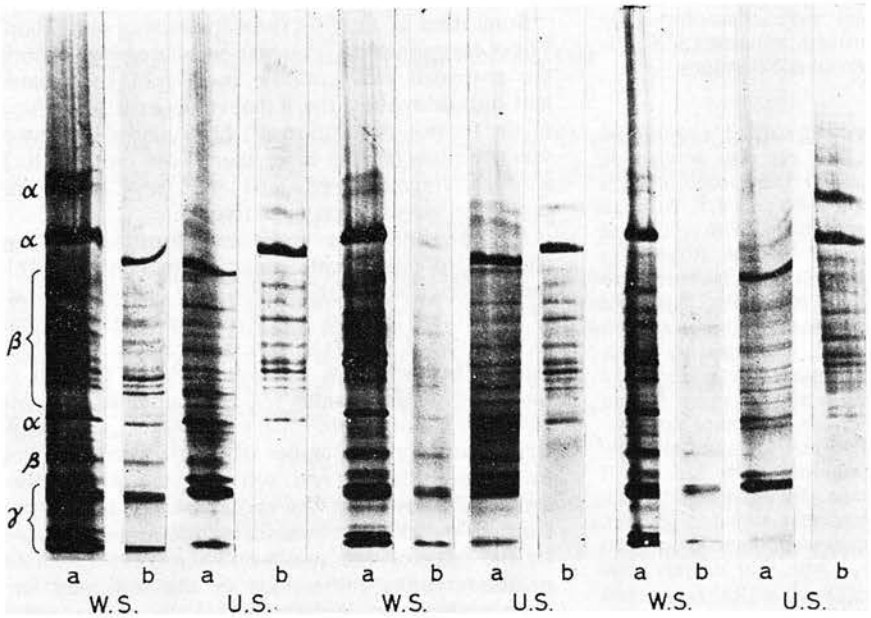


FIG. 2 *Isoelectric focusing in the presence of 6 M urea of the water-soluble (WS) and urea-soluble (US) protein fractions of the intact lenses* (a) *and the sonicated lens aspirates* (b) *of the second three rabbits*

those of the intact lenses in the γ and β regions, but they are also different from those of the aspirates of the first three lenses in that the total number of bands is reduced because of the loss of some γ- and β-crystallin subunits. The differences in the banding patterns of the urea-soluble fractions are the same as for the first three rabbits.

Discussion

While it is clear from these results that ultrasonication does affect the protein profile of treated lenses, the exact nature of the effect remains in question. The water-soluble fraction of the lens residue appears to be normal, but it is evident that the loss of β- and γ-crystallins from the aspirates increases as the degree of disintegration is more complete. However, the urea-soluble fraction, not only of the aspirate but also of the remaining lens material, is strongly affected, and, in addition, new material of an acid pI appears in the aspirate. These data suggest that sonication causes an overall transfer of β- and γ-crystallins out of the urea-soluble fraction—possibly into the water-soluble fraction, but more plausibly into the urea-insoluble fraction, since there is no corresponding increase of β- and γ-crystallins in the water-soluble fraction of the lens residue.

These two sets of data taken together imply that the probe causes physico-chemical changes which are very widespread, although the areas where complete disintegration has occurred may be very localized and depend upon the placing of the probe.

The loss of β- and γ-crystallins may be due to two processes. One of these is possibly additional cross-linking and insolubilization due to heating or other effects (in this context, β- and γ-crystallins are likely to be more susceptible than α-crystallin because of their higher sulphydryl (SH) content). The second possibly involves leakage of these smaller molecules from a disintegrated cell. Analysis of the composition of the urea-insoluble fraction and a search for crystallins in the aqueous humour are both necessary to answer these questions. The material of low pI may correspond to cell membrane proteins which we have observed in other studies (Odeigah, 1977).

References

CLARKSON, D.McG., and PHILLIPS, C. I. (1975) *Trans. ophthal. Soc. U.K.*, **95**, 477
—— —— (1976) *Brit. J. Ophthal.*, **60**, 759
CUTHBERT, J., CLAYTON, R. M., PHILLIPS, C. I., TRUMAN, D. E. S., and BARTHOLEMEW, R. S. (1978) "Analysis of the crystallin composition of individual Human Lenses: Characteristic modifications associated with different cataracts". *Interdisciplinary Topics in Gerontology*, in press
LIEM-THE, K. N., and HOENDERS, H. J. (1974) *Exp. Eye Res.*, **18**, 143
ODEIGAH, P. G. C. (1977) "Cell Surface Studies in Normal and Abnormal Chick Lenses". Ph.D. Thesis, University of Edinburgh
PHILLIPS, C. I. (1975) *Brit. J. Ophthal.*, **59**, 743
—— and WANG, M. K. (1971) *Ibid.*, **55**, 361
TRUMAN, D. E. S., and CLAYTON, R. M. (1974) *Exp. Eye Res.*, **18**, 485

HEADLEY BROTHERS LTD
109 Kingsway London WC2B 6PX
and Ashford Kent

Ageing of the Lens,
F. Regnault, O. Hockwin and Y. Courtois, eds.

ANALYSIS OF INDIVIDUAL CATARACT PATIENTS AND THEIR LENSES: PRELIMINARY OBSERVATIONS ON A POPULATION BASIS.

R.S. Bartholomew,[1] R.M. Clayton,[2*] J. Cuthbert,[2**] J. Duffy,[3] C.I. Phillips,[1] J.McK. Reid,[1] J. Seth,[4] D.E.S. Truman,[2] C. Wilson,[1] and S-H. Yim.[3***]

1. Department of Ophthalmology, University of Edinburgh, Princess Alexandra Eye Pavillion, Royal Infirmary, Chalmers Street, Edinburgh 3.
2. Department of Genetics, University of Edinburgh, King's Buildings, Edinburgh 9.
3. Department of Statistics, University of Edinburgh, King's Buildings, Edinburgh 9.
4. Department of Clinical Chemistry, University of Edinburgh, Royal Infirmary, Edinburgh 1.

SUMMARY

1. Distinct subgroups of cataracts were found, as defined by different relative proportions of the polypeptide chains of the lenses. Diabetic cataracts of whatever morphopathology differ in the relative proportions of their polypeptides from all other types of cataract, and cannot therefore be due merely to accelerated non specific senile changes.
2. Compared with age matched controls, cataract patients have significantly higher levels of plasma urea and glucose, and lower levels of albumin, total calcium and cholesterol. Most of the increase in plasma fasting glucose is due to the presence of a diabetic subgroup in the cataract patients.
3. The levels of plasma total calcium and albumin fall with increasing depth of colour in nuclear cataracts and with increasing maturity in cortical cataracts.
4. Cataract patients have a significantly higher incidence of glaucoma and uveitis.
5. Control patients have a significantly higher incidence of arcus senilis.
6. Cataract patients have a significantly higher usage of anti-hypertensive drugs.
7. There is a significant excess of topical drug application among cataract patients.
8. Cataract patients have a significantly higher incidence of endocrine disorders.

* To whom reprint requests should be addressed.
** In partial fulfilment of the degree of Ph.D.
*** In partial fulfilment of the degree of M.Sc.

INTRODUCTION

It is a regular feature of diagnostic analyses that a syndrome may be associated with several separate underlying aetiologies and that inability to discriminate between them hinders both investigation and the possible development of rational therapy or preventive measures.

Cataract is classified by the location of the opacity within the lens (e.g. nuclear or cortical) or by time of onset - (e.g. congenital or senile cataracts). A small number of cataracts have been specifically distinguished by their regular association with non-ocular pathology; the very few cataracts for which medical rather than surgical treatment has proved possible are from this group. It would be reasonable to expect, therefore, that a method for distinguishing accurately cataracts that are at present classified together on the basis of morphopathological criteria would permit further specific and directed investigations and eventually lead to an enlargement of the list of cataracts susceptible to non-surgical intervention or prevention.

Furthermore, an investigation into the conditions which predispose to cataract or which constitute a liability in certain circumstances should be investigated with the eventual aim of designating conditions conferring significant risk to definable subgroups within the population.

This report describes some preliminary results from a survey on cataracts in South East Scotland. On the one hand we have continued our investigations on biochemical profiles of individual cataractous lenses (1,2) in order to determine the validity of biochemical subgroups, and on the other collected information on the patients and on a control population matched for age and sex. with the eventual aim of seeking associations between ophthalmologically and biochemically defined subgroups with factors from the epidemiological, medical and clinical records. The associations are sought by stepwise discriminant analyses for the protein profiles, and by univariate methods for the epidemiological and other factors. The factors chosen for recording

Figure 1. Sheets 1 and 2 of Patient record card 1, Epidemiology and Medical Records. (59 items). Each of 5 record cards covers a number of items each with a range of coded values or alternatives.

CATARACT ANALYSIS AND EPIDEMIOLOGY — Coding Schedule

Card Number — 1 — 1

Case Number
Col. 2 3 4 5 6 7 8 9 10 11
No.

Experiment Serial Number
Col. 12 13 14 15

Status/Patient Control Relative
1 2 3 — 16

Year of Admission to Trial — 17 18

Month of Admission
1 Jan 7 July
2 Feb 8 Aug
3 Mar 9 Sept
4 Apr 10 Oct
5 May 11 Nov
6 June 12 Dec — 19

Date of Month of Admission

Sex N/K Male Female
12 1 2 — 20 21 22

Age — 23 24

Age group
12 N/K
1 0-9 6 50-59
2 10-19 7 60-69
3 20-29 8 70-79
4 30-39 9 80-89
5 40-49 10 90+ — 25

Occupation Physical Hazard (specify)
11 NA 12 NK 0 None
1 Trauma - particles (e.g. mining, inthe operator, quarrying)
2 Trauma - Other (e.g. diving).
3 Heat - (e.g. Foundry, glass blowing, potter).
4 Light - (e.g. Welding, microscopy).
5 Radiation - Other, (e.g. microwave ovens, communications, engineering, radar, X-ray).
9 Other, specify. — 26

Occupation Chemical Hazard (specify)
11 NA 12 NK 0 None
1 Organic solvents (paint, dry cleaner etc).
2 Anaesthetist
3 Brewing/barman
4 Chemical manufacturing, petroleum
5 Fertiliser or crip spraying
6 Ashphalting, caulking, etc.
9 Other, specify — 27

Occupation Other Hazard
11 NA 12 NK 0 None
1 Exposure to human infections
2 Exposure to animal infections
9 Other, specify — 28

Employment CODOT code
State occupation of Individual
NA 11 NK 12 — 29 30 31 32 33

Status CODOT code
State occupation of head of household
NK 12 — 34 35 36 37 38

Origin 12 NK
1 British Isles
2 Europe
3 Africa 15 years UK
4 " first generation
5 India 15 years UK
6 " first generation
7 Other (specify) 15 years in UK
8 As 7, first generation — 39

CATARACT ANALYSIS AND EPIDEMIOLOGY — Coding Schedule

Card Number — 1 — 1

Experiment Serial Number — 12 13 14 15

Status/Patient, Control Relative
1 2 3 — 10

Domicile, Location
12 NK
1 Edinburgh & Lothian
2 NW Scotland
3 NE "
4 SE "
5 SW "
6 Rest of Britain
7 Immigrant - recent
8 Immigrant - over 18 years
9 Other (specify) — 40

Domicile, type
12 NK
1 Urban
2 Rural
3 Institutional/e.g. asylum, prison, orphanage.
4 Services, merchant navy
5 Industrial estate (rural industrial)
6 No fixed address — 41

Weight, Kg. — 42 43 44

Weight, cm. — 45 46 47

MEDICAL HISTORY
Serious infective illness - viral.
1 Yes, specify
0 None 11 NA 12 NK — 48

Serious infective illness - bacterial
1 Yes, specify
0 None 11 NA 12 NK — 49

Medical conditions & treatment
Cardiovascular
11 NA 12 NK 0 None 1 Untreated
2 Digitalis 8 Other, specify
3 Warfarin 9 Multiple
4 Antihypertensives
6 Surgical — 50

Hepatic
11 NA 12 NK 0 None 1 Untreated
2 Drug treatment (specify)
3 Surgical
4 Other, specify
9 Multiple — 51

Renal
11 NA 12 NK 0 None 1 Untreated
2 Drug (specify)
3 Surgical
6 Other (specify)
9 Multiple — 52

Dermatological
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
3 Irradiation
8 Other (specify)
9 Multiple — 53

Endocrinological EXCLUDING DIABETES
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
3 Irradiation
4 Surgery
6 Other (specify)
9 Multiple — 54

Diabetes
11 NA 12 NK 0 None 1 Untreated
2 Diet alone
3 Oral hypoglycaemics
4 Insulin
9 Multiple — 55

Age of Onset of Diabetes
11 NA 12 NK
0 0-9 years 4 40-49 years
1 10-19 years 5 50-59 years
2 20-29 years 6 60-69 years
3 30-39 years 7 70-79 years
8 80+ years — 56

CATARACT ANALYSIS AND EPIDEMIOLOGY

Refraction before onset of cataract — 31 32 33
0 = Emmetropic

Refraction at presentation — 34 35 36
0 = Emmetropic

Years between first record of cataract and operation.
NK 12, NA 11, 0-1 = 0; 2-5 = 1; 6-10 = 2; 11-15 = 3; 16-20 = 4; 21 + = 5 — 37

Classification of Cataract (clinical appearance)

Nuclear
NK 12, NA 11, White 1, Yellow 2, Brown 3, Red/brown 4, Black 5. — 38

Cortical-Anterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, multiple 9. — 39

-Posterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9. — 40

-Peripheral
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 — 41

-Lamellar
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 — 42

-Sectorial
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 — 43

-Sutural
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 — 44

-Cuneiform
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9 — 45

Maturity
NK 12, NA 11, Not mature 1, Mature 2, Hypermature 3. — 45

Other
NK 12, NA 11, Blue dot 1, White dot 2, Coronary 3, bd + cor. 4, bd + wd + cor.7, wd + cor. 5, bd + wd 6. — 46

Distortion of Lens
NK 12, NA 11, Pseudoexfoliation 1, Displacement 2. — 47

LEFT EYE
Glaucoma NK 12, NA 11, No 0, OA 1, CA 2, Both 3, Secondary 4. — 48

Uveitis
NK 12, NA 11, No 0, anterior 1, posterior 2, both 3. — 49

Macular Degeneration
NK 12, NA 11, No 0, Yes, 1, (specify) — 50

Hypertensive Retinopathy
NK 12, NA 11, No 0, Yes 1, (specify) — 51

Diabetic Retinopathy
NK 12, NA 11, No 0, Yes 1, (specify) — 52

Retinal Detachment
NK 12, NA 11, No 0, Yes 1 — 53

Physical Injury
NK 12, NA 11, No 0, Perforating 1, Concussion 2, Radiation 3, Multiple 4. — 54

Other Eye Conditions
(Ophthalmos) ARCUS
e.g. (Microphthalmia) slight 2
(Keratoconus) mod. 3
severe 4
NK 12, NA 11, No 0, Yes 1, (specify) Multiple 5, (specify) — 55

Refraction before Onset on Cataract
0 = Emmetropic — 56 57 58

2a.

CATARACT ANALYSIS AND EPIDEMIOLOGY

Lens Protein Analysis

Coding Schedule

CARD NUMBER — 1

Expt. Serial Number — 2 3 4 5

Fraction
Water soluble 1
Urea soluble 2
Membrane 3 — 6

Densitometer Values

I_1 — 7 8

II_1 — 9 10

II_2 — 11 12

II_3 — 13 14

III_0 — 15 16

III_1 — 17 18

III_2 — 19 20

III_3 — 21 22

III_4 — 23 24

IV_1 — 25 26

IV_2 — 27 28

V_1 — 29 30

V_2 — 31 32

VI_1 — 33 34

Cataract Description
White 1, Brown 3, Yellow 2, NA 12 — 35

Cortical
Mature 1
Hypermature 2
Ant. Pol. 3
Post. Pol. (Cupuliform) 4
Multiple 5
Cuneform 6
NA 12 — 36

Blue Dot 1
White Dot 2
Coronary 3
Multiple 4
NA 12 — 37

Vesicles & Clefts 1
Granules 2
Pseudo Exfoliation 3
Multiple 4
Other (specify) 5
NA 12 — 38

Diabetic 1
Corticosteroid 2
Juvenile 3
Mongol 4
Coppock 5
Myotonic 6
Other (specify) 7
NA 12 — 39

2b.

Figure 2.

were based on the information in the literature which is outlined below.

Interaction of Factors Conferring Liability to Cataract

The synergism of cataractogenic factors has received special consideration from Koch, Hockwin and colleagues (3,4), they term the potentiating interaction of a cataractogenic factor with another factor not in itself a cataractogen cocateractogenesis, and distinguish this from syncataractogenesis, in which factors not directly cataractogenic, or at subcataractogenic level, (called by these authors 'subliminal') may, together, give rise to cataract.

For example, the effect of UV light is potentiated by inhibitors of catalase, (5) and the glucocorticoid prednisone has a potentiating effect on galactose cataracts in rats (6). Certain medical conditions may confer especial susceptibility to cataractogenic agents. Corticosteroids may accelerate senile cataract development in kidney transplant patients and azathioprine may have a potentiating effect (7). Corticosteroid cataract also occurs in patients with rheumatoid arthritis but not in those with asthma,(8). Interacting factors need not produce the same immediate effects on the lens: X-rays and galactose feeding show synergism, but each alone produces a different modification of the protein pattern of the lens (9).

Epidemiology of Cataracts

Senile cataract may be said to be the rule in advanced age. 50% of all persons in the 6th decade, and virtually everyone by 80 years of age have some opacity, (10,11). Congenital cataracts were one of the four major causes found of visual handicap in a population of children in Holland (12). Operations for cataract are much more numerous than for any other ophthalmological condition (13,14,15). About 75% are of the senile type.

Figure 2a. Sheet 2 of patient record card 2, Ophthalmological Record (57 items). 2b. Card 5, sheet 1 of Lens Protein Analysis (39 items).

Card 3 covers Plasma Analyses (19 items) and Card 4, further information on individual lenses (21 items).

Fig.2a, for example, codes for 23 items.

Striking geographical and ethnic differences in the prevalence of cataract are found, but direct comparisons between figures should be regarded with caution, as differences will arise owing to different definitions of blindness, incomplete registration or even none at all. The percentage of cataract amongst those registered blind ranges from 3.6% in Canadian Eskimoes (16), 16% - 20% in the U.S.A. (17,18), 22.6% in Britain (19), 35% in Nigeria (20), 40% (18) and 10 million people (21) in India and Pakistan and 45% in Kenya (22). Even within a country, areas and groups differ. Mann, (23) found the incidence of blindness registrations in Natal, South Africa, to be 10% in Europeans, 15-16% in Bantu and 33% in Indians. Chatterjee (24) found an incidence of advanced cataract that varied from 1.5 - 7.2% of the whole population of 5 areas in the Punjab, (in general, advanced cataracts are present at an earlier age in Africa and Asia than is found in Western countries).

Genetic factors, and differences in climate, irradiation and nutrition have all been proposed as possible causative factors for this wide range of incidences and of age of onset of cataracts. Domicile and ethnic origin and social group are therefore amongst the factors which we record (fig 1).

Association with Occupations

Cataract following exposure to ionising radiations, such as X-rays, is well known, and the near ultra violet has been suggested as possible cause for the higher incidence of cataract in tropical climates, and in outdoor workers; and to account for the observation that the lower quadrant of the human lens is often the first to show cataract formation (25).

Heat cataract from infra-red radiation was legally established as an occupational disease, (26): and high levels of longer wave irradiation (diathermy and microwave) can produce rapid appearance of cataract (27,28) while continued sub-threshold doses of microwaves, such as occur in certain occupations are associated with liability to cataract, (29,30,31,32). Many drugs and chemicals are known to produce cataracts in experimental animals, and in humans after exposure or systemic absorption. We have therefore recorded occupation (fig.1) classified by the nature of the hazard, (physical, chemical, etc).

Association with other Diseases

Selective epidemiological investigations have been undertaken where a cause for cataract is already known or suspected. For example, there is no increase in the total prevalence of cataract in diabetics over 40 years of age but once cataract is present progression is more rapid (11,14) Acute angle closure glaucoma is associated with increased incidence of cataract (33), and statistical associations with blood groups have been reported (34), and other disease associations have also been reported (7)

We have recorded a wide range of disease conditions in our population, and a series of assays of plasma levels of various components (fig.2) normally regarded as indicative of renal, hepatic, endocrine and other dysfunctions.

Medication

Certain drugs contribute to the development of lens opacity in man, including the anti-neoplastic agent Busulfan, corticosteroids and their derivatives such as Dichlorisone, weight-reducing drugs formerly in use such as Dinitro-o-cresol and Dinitrophenol, many miotics such as pilocarpine, Triparanol, (an agent formerly used to treat hypercholesterolaemia), and the tranquiliser 1-4-bis (phenylisopropyl)-piperazine (Quietidin). These together with other substances tested on animals and found to be cataractogenic, have been reviewed in detail (35). Miotics and especially cholinesterase inhibitors, such as diisopropyl fluorophosphate (Floropryl), paraoxon (Mintacol). demacarium(Tosmilen, Humorsol,) and echothiophate (Phospholine iodide) can be cataractogenic (36). Among antibiotics, polymyxin B has been found to be cataractogenic in rats (37), and among substances which induce opacities in the cultured lens are phenacetin and sulphonilamide (38). We have therefore recorded any drug used continuously for four months or more.

Genetic Factors Conferring Liability to Cataract

Genetic factors are found associated with cataract in man and in several species of animals (39,40,41). Genetic factors may be involved in senile

cataract (42). The clearest cases are those associated with several specific although rare syndromes or metabolic diseases; for example galactosemia and galactokinase deficiency, the mannosidoses, some of the amino-acidureas, pseudohypoparathyroidism, Lowe's Syndrome and a few others. The discovery of a rare mutant may be regarded as an indication of the existence of an hitherto unrecognised locus concerned with a particular regulatory function in metabolism or development (43). Virtually all loci, if biochemical investigation is possible, prove to have many alleles, some fully effective under all circumstances examined, some of severely impaired efficiency under all circumstances examined, and some associated with a liability to deleterious expression only in the presence of certain environmental conditions or specific drugs and other agencies. For example, 80 alleles of G6-P-D in man were classified in this way (44), and indeed, cataract has been found associated with a member of this allelic series while lens G6PD is deficient in individuals with alleles conferring primaquine sensitivity (45,46).

The genotype may modify the expression of cataractogenic agencies: for example aldose reductase deficient mice are resistant to diabetic cataract (47) and pigmented rats develop naphthalene cataracts earlier than albinos (48). The increasing body of evidence for pharmacogenetic variants in man (49) suggests that there are very likely to be familial differences in the rate of metabolism of cataractogenic drugs which would affect the likelihood of a treated individual developing cataract. Finally there are certain associations which merit further inquiry. High levels of galactose are cataractogenic in experimental animals (50) but infants homozygous for either uridyl transferase deficiency or galactokinase deficiencies develop cataracts unless on a completely galactose-free diet (51). For these reasons we have recorded the existence of known genetic pathology and the presence of cataract in consanguineous and non consanguineous relatives.

The Analysis of Crystallin Composition as a Diagnostic Procedure for Specific Cataracts.

Changes in the crystallin content of cataractous lenses are well attested (52). Cataracts may be divided into nuclear cataracts, further sub-divided by colour, or cortical, which may be described by area, or degree of involvement (posterior, anterior, peripheral, mature, hypermature) and further described by the appearance of the lesion (thus, vacuoles, clefts, granules, etc.). The site and nature of the lesion must be reflected by the changes in the crystallin profile ; since the crystallin balance differs according to the region of the lens, and may also be modified by changes in the cell membranes, in growth conditions (including available metabolites, ionic concentrations, etc.) by genotype, and other factors (reviewed 1,53,54). Our reasons for analysing cataractous lenses individually (fig.2), instead of pooled material have been fully discussed elsewhere, together with some preliminary results (1).

Materials and Methods

1. Lens Analyses All lenses are placed on solid CO_2 in the operating theatre immediately after extraction, and stored in liquid nitrogen until processed for analysis.

A. Proteins The proteins of the water soluble and the urea soluble phases of each lens have been resolved by isoelectric focussing in dissociating conditions and the relative proportion of the total protein in each peak measured by an integrating densitometer. The methods employed have been fully described elsewhere (1). Analysis of other measurements (wet weight, dry weight, total carbohydrate, composition of urea insoluble residue, etc.) are still in progress and will be described elsewhere.

B. Statistical Procedures for Lens Analyses. Stepwise discriminant analyses were performed for the protein profiles for water soluble and urea soluble phases of each lens; of the various different cataract types using the B.M.D.

program package (55). The full details of the procedures employed will be published elsewhere.

2. Statistical Analysis of Population Data Univariate analyses of data from patients and controls have been performed using S.P.S.S. (56). Since data collation is at a relatively early stage and many of the comparisons involve relatively rare features, results are reported which achieve significance at the 5% level; clearly therefore these must be treated with some caution, the indicated significance level being a valuable guide. Numbers of patients and controls may vary between tables because missing values have been excluded from analysis.

3. Biochemical data Blood was collected from patients and controls under conditions standardised as far as possible to eliminate any errors which might be attributable to venous stasis, or posture. Plasma was analysed using a Technicon Sequential Multiple Analyser and Computer (S.M.A.C.), using standard analytical methods.

RESULTS AND DISCUSSION

Protein Profiles of Individual Lenses

The conclusions that may be drawn are the following: there are indeed a series of distinct subclasses of cataract as defined by their protein profiles (2, and fig.3). This confirms the conclusions we drew previously, based on a visual comparison of profiles and an inspection of values for each peak (1). The results for the water soluble fraction do not parallel those for the urea soluble in any case (2). The different variables are important in themselves. There is no variable which is most or least useful in all discriminations (2). Different variates are important according to the particular comparison made.

The degree of divergence between groups of cataracts varies. In the case of urea soluble fractions, brown nuclear plus mature cortical cataracts are significantly different at the 5% level from the following: pure brown

nuclear, mature cortical, brown nuclear with cuneiform cortical, brown nuclear with posterior polar, and brown nuclear with multiple cortical scoring. Comparing water soluble fractions for example brown nuclear with cortical involvement, brown nuclear with posterior polar differs from brown nuclear with cuneiform cortical involvement, at the 5% level. Both water and urea soluble fractions of diabetics differ significantly (at the 2.5% level) from the equivalent fractions of all non diabetic cataracts.

Although there are general similarities in shape overall between the densitometer traces for yellow and brown nuclear cataracts respectively, (1), the statistical analysis of the water soluble fractions reveals that they are significantly different. The means for these two types are distinct and the populations show no overlap (fig. 3a,b). This difference is highly significant, either there is a threshold in the transition from yellow to brown, alternatively the formation of a brown cataract requires a specific step not operative in cataracts that remain yellow.

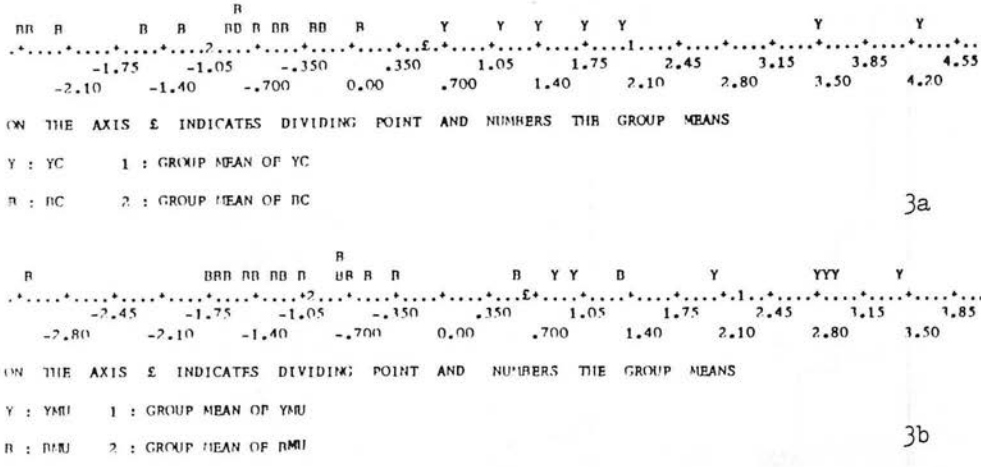


Figure 3

Computer-generated print-out of comparisons, by stepwise discriminant analusis, of the quantitative polypeptide chain composition of the water soluble fraction of cataractous lenses. 3a, YC, yellow nuclear plus cuneiform, BC, brown nuclear plus cuneiform. 3b, YMU, yellow nuclear multiple cortical involvement, BMU brown nuclear, multiple cortical involvement.

A comparison may cause unexpected groups to emerge. Two Mongol, (Down's Syndrome) cataracts were previously found to exhibit a unique profile (1). When these were compared statistically against the non Mongol population a group of eight lenses clustered together at the extreme end of the distribution together with the Mongols. These were identified and their records examined. All eight had high wet weights and low dry weights, and four were probably congenital anomalies, or at least of early origin.

Specific components may be compared. Fig. 4 a, b, and c represent frequency histograms of a single component. 4a shows that juvenile cataracts are not in the normal distribution for the value of one of the γ crystallins, which is compared in 4b for nuclear and cortical cataracts. 4c shows that different cataracts form distinct although variable populations with respect to a β crystallin.

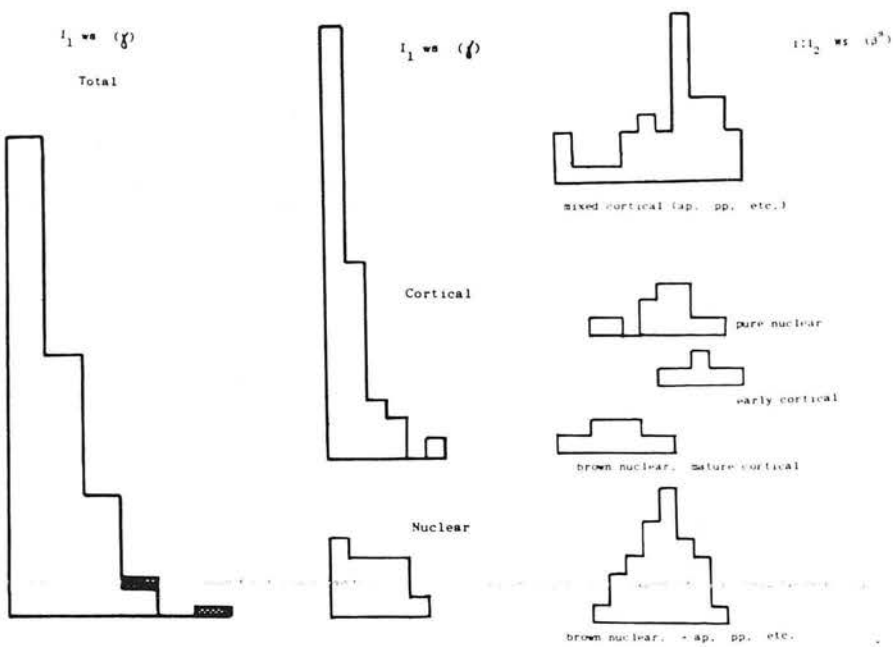


Figure 4.

Frequency histograms for the quantitative levels of a specific polypeptide chain in different types of cataract. 4a) γ crystallin (subunit I(1)) 98 cataracts, juvenile cataracts cross hatched. 4b) 38 cortical cataracts, 15 nuclear cataracts. 4c) β^S crystallin, (subunit III$_2$) 59 mixed cortical cataracts. 12 pure nuclear, 12 early cortical, 9 brown nuclear mature cortical, 32 brown nuclear multiple cortical.

Component 3(1) in the urea soluble phase (1) is absent in about 50% (7/16) of diabetic cataracts of varied morphopathology, but in about 10% (22/210) of non diabetic cataracts. The same component in the water soluble phase was absent in about 40% (6/15) of diabetic cataracts and in about 5% (7/146) of non diabetics. (A number of patients were found to be undisclosed diabetics). Other differences were also found: for example 10 diabetic lenses of widely varied morphopathology but with cupuliform involvement, differed from 37 diabetic lenses without cupuliform involvement ($P < 0.01$), the values being 41% (s.d. 6.7) and 29.8% (s.d. 10.3) respectively. Of 179 non diabetic lenses, 58 with cupuliform involvement had 32.2% dry weight (s.d. 9.4) and 121 without such morphology had 27.8% dry weight (s.d. 11.0). Diabetic lenses with cupuliform involvement seem a special group, and this suggests that cupuliform pathology may not have a uniform biochemical significance. The finding that diabetic cataracts of whatever morphopathology have special features is not compatible with the currently accepted view that diabetes merely accelerates the formation of a senile cataract.

Although biochemically defined subgroups have emerged from this analysis, it is not possible to form hypotheses at present regarding the relationship between morphopathology and the relative susceptibilities of the different crystallins to loss or insolubilisation.

The Patient and Control Groups

These results represent only a preliminary screening of our population of some 300 individuals. (Transformations will be applied where necessary before any final analysis, and the data from the much larger population we now have will be incorporated).

Some observations cannot yet be interpreted. For example the incidence of cataract in non consanguineous relatives of controls is 9% but of patients 0.8%. We must ascertain what proportion of controls are motivated to volunteer because their spouse has cataract.

Our results confirm the previous findings on the association of cataract with uveitis (7.3% of patients, 1.2% of controls, $P < 0.025$), glaucoma (13.8% of patients, 0.6% of controls, $P < 0.0001$) and diabetes (patients 12.3%, controls 0%, $P < 0.0001$). Cataract is also associated with topical application of drugs (6% compared to 0.6%, $P < 0.025$), these included miotics which have previously been implicated (35,36), and corticosteroids (of 8 cases in 127 patients, 2 used miotics, 3 corticosteroids, and 3 used both 1 case in 155 controls used adrenalin).

No significant differences have emerged so far between patient and control groups in the incidence of respiratory, renal or cardiovascular disease, but genetic endocrine conditions were found more often in patients, (6.6% and 0.7% respectively, $P < 0.025$).

There were no significant differences in the use of diuretics, corticosteroids, anticonvulsants, antibiotics, vasodilators, or tranquilisers, (larger numbers will be required to assess possible assymetric distributions of specific drugs within a general class). There was however a significant difference in the use of antihypertensive drugs as a treatment for cardiovascular disease (Table 1). The incidence of hypertension is being assessed and will be reported elsewhere.

TABLE 1

Cardiovascular Conditions and Treatment

	Patients	Controls		
No cardiovascular condition	105	141	246	
Anti-hypertensive drugs	8	1	9	$P < 0.025$
Other drugs	23	24	47	
	136	166	302	

The biochemical assays on plasma consituents of 138 patients and 167 controls are shown in Table 2, and components showing significant differences between 16 diabetics and 289 non-diabetics in Table 3.

TABLE 2

Plasma Constituents of Patients (P) and Controls (C)

		Mean (s.d.)		Mean (s.d.)	Significance
UREA(m.mol/l)	P	5.81 (2.06)	C	5.36 (1.29)	0.0204
CO_2(m.mol/l)	P	27.55 (3.40)	C	28.44 (2.34)	0.0073
ALANINE AMINOTRANSFERASE (u/l)	P	14.48 (16.70)	C	20.91 (31.02)	0.0297
TOTAL CALCIUM (m.mol/l)	P	2.39 (0.119)	C	2.46 (0.103)	<0.0001
CHOLESTEROL(m.mol/l)	P	6.03 (1.42)	C	6.66 (1.23)	<0.0001
ALBUMIN (g/l)	P	40.80 (3.60)	C	43.13 (2.24)	<0.0001
TOTAL PROTEIN (g/l)	P	69.67 (5.82)	C	73.14 (4.61)	<0.0001
PLASMA FASTING GLUCOSE(m.mol/l)	P	6.08 (3.50)	C	4.76 (0.54)	<0.0003

No significant difference overall: Na^+, K^+, bilirubin, alkaline phosphatase, inorganic phosphate, creatinine, urate, aspartate aminotransferase, triglycerides.

TABLE 3

Plasma Constituents of Diabetics (D) Vs Non-Diabetics (ND)

D, 16, ND, 289 Means, (s.d. in brackets)

		Mean (s.d.)		Mean (s.d.)	Significance
UREA	ND	5.51 (1.59)	D	6.91 (3.08)	0.0015
PLASMA FASTING GLUCOSE	ND	4.95 (0.83)	D	12.90 (7.42)	<0.0001

Lenses with cortical cataract contain grossly elevated levels of free and bound calcium (57,58) especially in fully opaque cortical cataracts. Levels in nuclear cataracts are less well clarified: they were found to be normal in pooled nuclear cataracts (57,59) or at least very variable (60), but there is evidence that brown nuclear cataracts contain higher levels than yellow nuclear cataracts (59,60).

In contrast to these reports of raised calcium in lenses with cataract, we find depressed levels in the plasma of cataract patients overall, with a fall with increasing depth of nuclear colour, while within the cortical group, the levels also show a declining trend with increasing severity of involvement.(Tables 4 and 5). The lowest levels were found in the 3 hypermature cataract patients. In the diabetic group, the means for both calcium and albumin are similar to those of the mature cortical and yellow nuclear groups.

TABLE 4

Nuclear Cataracts: Plasma Constituents. Means (s.d. in brackets)

	No Nuclear Involvement	Nuclear Cataract				
		White	Yellow	Brown	Red-Brown	Significance
Ca^{++}	2.41 (0.117)	2.45 (0.116)	2.38 (0.110)	2.37 (0.119)	2.37 (0.139)	0.0383
ALBUMIN	42.5 (3.15)	43 (3.04)	40.7 (3.16)	39.3 (3.42)	37.7 (5.13)	< 0.0001
TOTAL PROTEIN	71 (5.0)	72 (5.3)	71 (6.2)	68 (5.4)	62 (5.0)	0.0010
UREA	4.65 (1.66)	5.23 (1.66)	5.78 (1.80)	5.47 (2.34)	5.50 (1.74)	0.0132

TABLE 5

Cortical Cataracts: Plasma Constituents

	Early	Mature	Hypermature	Not significant at 5%
Ca^{++}	2.42 (0.123)	2.40 (0.106)	2.33 (0.611)	Trends only
ALBUMIN	41.7 (3.3)	40.8 (3.2)	38.7 (47)	0.0339
ALKALINE PHOSPHATASE	88.76 (43.3)	163.65 (374.4)	85.67 (45.1)	

Both the optical appearance and the anomalous values for calcium and albumin for white nuclear cataracts imply differences from the other forms of nuclear cataract.

The trends in plasma total calcium within both cortical and nuclear groups were associated with parallel trends in albumin, the latter largely accounting

for the lower total protein in the cataract group as a whole. The association of hypocalcaemia and cataract is known (61,62). The trend in total calcium within the nuclear and cortical groups appears to be largely secondary to the changes in albumin concentration. However, within the cataract group as a whole, the lower albumin concentration appears to account for only part of the lower calcium levels, and other causes of the tendency to lower total calcium levels cannot be excluded.

An excess of renal disease, for example, could account for the relatively lower total calcium and albumin levels, and higher urea levels in the cataract group, and would be consistent with reported evidence of transient cataract associated with temporary renal dysfunction, (63) and of cataract with renal failure (64,65). However, the lack of significant difference in (a) plasma creatinine and urate levels and (b) incidence of overt renal disease between cataract and patient groups suggests that mild renal disease is unlikely to be an important factor in causing the differences in calcium, albumin and urea levels. Nephropathy might be expected in the diabetic group, but although their plasma urea is elevated, it does not implicate renal dysfunction as an important factor for the calcium levels, as the mean for total calcium and albumin in diabetes are similar to those for the mature cortical and yellow nuclear groups, and it will be noted that the brown nuclear and hypermature cortical groups have even lower levels.

General dietary deficiency might account for the low total calcium and albumin in the cataract group, but would not be consistent with the higher urea levels. There is no biochemical evidence of differences in hepatic function between the two groups that might account for the differences in albumin levels. Although medication might be invoked to rationalise the biochemical differences, (for example, an excess of anticonvulsant users in the cataract group as a whole might contribute to the lower calcium levels in these groups, or excess of protein catabolic drugs, such as

corticosteroid, which might account for the low albumin and raised urea), in fact no significant differences between patients and controls have yet emerged in the use of such drugs. The difference between patients and controls in plasma fasting glucose (6.0 and 4.8 respectively) appears to be due to the diabetics: if these are removed, the mean for the cataract patients (5.2) is higher than that for controls but not significantly so.

Two other features of a population with an excess of diabetics are not found, triglyceride levels do not differ significantly between patients and controls, and cholesterol is actually lower in patients than controls, ($P < 0.0001$), the reverse of expectation. Possible explanations for the latter would include higher levels of thyroid function in patients (not measured) or an increase of primary hyperlipidemia in controls; however unlikely this may seem, it will be noted from Table 6 that there is also an excess of arcus senilis in controls ($P < 0.0001$), which would be in agreement with higher cholesterol. As with the calcium levels, an inverse relationship between serum and lens levels seems to occur; cataractous lenses are reported to contain elevated levels of cholesterol (66,67).

TABLE 6

Arcus Senilis Patients (P) Vs Controls (C)

	P	C		
ABSENT	109	90	119	
SLIGHT	18	51	69	$P < 0.0001$
MODERATE AND SEVERE	11	23	34	
	138	164	302	

Total Co_2 levels differ only slightly, but one group, cataract patients with glaucoma, have a mean of 25.9 as compared to controls (28.45). Acetazolimide dosage may account for part of this difference. The high alkaline phosphatase levels and the extreme variability in the mature cataract

group suggests that bone metabolism might be affected in certain of these patients.

CONCLUSION

Although none of these data lead to unequivocal conclusions it would seem to us that the analysis of individual lenses is justified, in that the definition of different cataracts on a biochemical basis might on the one hand lead ultimately to a clearer understanding of the range of mechanisms leading to opacity, and on the other to the validation of subgroups of cataract selected for assessment of significant associations with different types of cataractogenic agencies. The emergence of unexpected differences (as with plasma total CO_2, and cholesterol) now indicates that certain specific drugs may require statistical investigation. It is necessary to evaluate whether it is the disease condition or the drugs employed which is associated with liability to cataract. The size of the population under investigation is being increased considerably, and we hope that some of the problems raised by the associations reported here may be clarified by inserting certain specific questions into our recording system. The inverse relationship found, both for calcium and for cholesterol, between levels in plasma and reported levels in cataractous lenses, is inexplicable at present but does suggest possible experimental approaches with animal models.

ACKNOWLEDGEMENTS

We are glad to thank the following surgeons for their cooperation in making patients and their lenses available for this survey: A. Adams, J. Hughes, G.T. Millar, and N. Stokoe.

We are also grateful to those colleagues who have either advised us on problems during the preparation of the proformas or who have agreed to act as medical consultants: A. Doig, R.S. Illingworth, J. Munro, J. Williamson.

We are grateful to the Scottish Home and Health Department, The W.H. Ross Foundation for the Study of the Prevention of Blindness and the Iris Fund for Prevention of Blindness for support. The work of the Edinburgh Lens Group is also supported by the M.R.C. and the C.R.C.

We are grateful to Mrs L. Dobbie for her patience and care in dealing with the manuscript.

REFERENCES

1. CUTHBERT, J., CLAYTON, R.M., TRUMAN, D.E.S., PHILLIPS, C.I. and BARTHOLOMEW, R.S. (1978) Interdiscipl. Topics Geront. 13, 183-192.
2. YIM, S.H. (1978) Discriminant analysis of the soluble protein composition of individual cataractous human lenses. M.Sc Thesis, University of Edinburgh.
3. HOCKWIN, O. (1976) Les Colloques de l'INSERM. 60, 263-279.
4. HOCKWIN, O. and KOCH, H-R. (1974) In Cataract and Abnormalities of the Lens. Ed. Bellows, J.G. Grune and Stratton, pp. 243-254.
5. KUCK, J.F.R. (1976) Docum. Ophthal. Proc. Ser. 8, 241-260.
6. BETTMAN, J.W., FUNG, W.E. and NOYES, P.O. (1964) Invest. Ophthal. 3, 678.
7. KOCH, H.R., HOCKWIN, O. and OHRLOFF, C. (1976) Metabolic Ophthalmol. 1, 55-62.
8. SUNDMARK, E. (1966) Acta Ophthal. 44, 291-297.
9. MANSKI, W., YORK, A.T., METTIAM, G.R., WORGUL, B.V. and SZECHTER, A. (1976) Docum. Ophthal. Proc. Ser. 8, 275-292.
10. FISCHER, F.P. (1948) Modern Trends in Ophthalmology 2nd Series. A.Sorsby Ed. p 54-70 Butterworth London.
11. McGUINNESS, R. (1967) Brit. Med. J. 11, 416-418.
12. SCHAPPERT-KIMMIJSER, J. (1973) J. Paediatr. Ophthal. 10, 226-232.
13. FUCHS, A. (1962) Geography of Eye Diseases. Vienna. Notring der Wissenschaftlichen Verbunde Osterreiche.
14. CAIRD, F.I. (1973) CIBA Foundation Symposium (New Series). Associated Scientific Publications - Amsterdam. 19, 281-296.
15. SORSBY, A. (1962) Exp. Eye Res. 1, 296-299.
16. WYATT, H.T. (1973) Canad. J. Ophthal. 8, 291-297.
17. KINSEY, V.E. (1967) Vision and it's disorders. U.S. Public Health Service. Bethesda.
18. SPECTOR, A. (1974) Invest. Ophthalmol. 13, 325-332.
19. SORSBY, A. (1966) Reports on Public Health and Medical Subjects. No.114 H.M.S.O., London.
20. OSUNTOKUN, O. and OLVRIN, O. (1973) Brit. J. Ophthal. 57, 27-33.
21. VENKATASWAMY, S. (1972) Jerusalem Seminar on the Prevention of Blindness in Causes and Prevention of Blindness. Michaelson, I.C. and Berman, E.R. (Eds.) New York, Academic Press Inc. p623.
22. BISLEY, G.G. (1963) E. Afr. Med. J. 40, 570-573.
23. MANN, I. (1966) Culture, race, climate and eye disease: An introduction to the study of geographical ophthalmology. C.C. Thomas, Springfield, Ill. pp316-317.
24. CHATTERGEE, A. (1973) CIBA Foundation Symposium 19, 265-271.
25. DUKE-ELDER, S. (1972) System of Ophthalmology. Vol. XIV Henry Kimpton London p1290.
26. LEGGE, (1907) Home Office Reports on Glassworkers, London.
27. MILROY, W.S. and MICHAELSON, (1972) Aerospace Med. 43, 67-75.
28. ZARET, M.M. (1974) N.Y. State J. Med. 74, 2032-2048.
29. GOLYCHEV, V.N. and NIKITINA, O.N. (1974) Vestn. Oftalmol. 6, 81.
30. McAFFEE, R.D., CAZENAVETTE, L.L. and HOLLAND, M.G. (1975) Amer. N.Y. Acad, Sci. 247, 135-141.
31. ODLAND, C.T. (1972) J. Occup. Med. 14, 544-547.
32. AURELL, E., and TENGROTH, B. (1973) Acta. Ophthal. 51, 764-771.
33. JONES, B.R. (1959) Trans. Ophthal. Soc. U.K. 79, 753-760.
34. MURTY, J.S. (1973) East. Arch. Ophthal. 1, 1-7.
35. GRANT, W.M. (1974) Toxicology of the Eye, 2nd Edition. C.C. Thomas Springfield, Ill.
36. AXELSSON, U. (1973) CIBA Foundation Symposium 19, 249-257.

37. COTLIER, E. and APPLE, D. (1973) Exp. Eye Res. 16, 69-77.
38. EDWARDS, A., GUPTA, J.D. and HARLEY, J.D. (1973) Exp. Eye Res. 15, 495-498.
39. KIRSCH, R.E. (1975) The Lens. Arch. Ophthal. 93, 284-314.
40. MERIN, S. (1975) In Genetic and Metabolic Eye Disease, ed. by Goldberg, M.F. Little, Brown & Co. pp.337-355
41. VAINISI, S.J. and GOLDBERG, M.F. (1975) In Genetic and Metabolic Eye Disease, ed. by Goldberg, M.F. Little, Brown & Co. pp215-231.
42. NETTLESHIP, E. (1905) Roy. Lond. Ophthal. Hosp. Rep. 16, 179-246
43. KREITMAN, N. and CLAYTON, R.M. (1974) In The Edinburgh Companion to Psychiatric Studies. Ed. A.D. Forrest., Livingston, Edinburgh. 402-406.
44. MOTULSKY, A.G., YOSHIDA, A. and STAMATOYANNOPOULOS, G. (1971) Ann N.Y. Acad. Sci. 179, 636-643.
45. WARBURG, M. (1975) 2nd Int. Symposium on Metabolic Eye Diseases. Edinburgh.
46. ZINKHAM, W.H. (1961) Bull. Johns Hopkins Hosp. 109, 206-216.
47. VARMA, S.D. and KINOSHITA, J.H. (1974) Exp. Eye Res. 19, 577-582.
48. KOCH, H-R., and DOLDI,K. (1975) Exp. Eye Res. 20, 180-
49. VESELL, E.S. (1973) Progr. Med. Genet. 9, 291-367.
50. KINOSHITA, J.H. (1974) Invest. Ophthalmol. 13, 713-724.
51. HARRIS, H. (1975) Principles of Human Biochemical Genetics. 2nd. Edition. North Holland, Amsterdam.
52. HARDING,J.J. and DILLEY, K.J. (1976) Exp. Eye Res. 22, 1-73.
53. CLAYTON, R.M. (1974) In The Eye Vol. 5 ed. Davson, H. Academic Press, London. pp399-494.
54. CLAYTON, R.M. (1979) In Mechanisms of Cell Change , J. Ebert and T. Okada (eds.) Wiley, N.Y. pp.129-167.
55. DIXON, W.J.W . (1977) Biomedical Computer Programs. P- series. Univ. of Calif. Press.
56. NIE N.H., HULL, C.H., JENKINS, J.C., STEINBRENNER,K. and BENT, D.H. (1975) Statistical package for the Social Sciences. 2nd Edition. McGraw-Hill.
57. DUNCAN, G. and BUSHELL, A.R. (1975) Exp. Eye Res. 20, 223-230.
58. BRACCHI, P.G., CAMPININI, G. and MARAINI, G. (1978) Interdiscipl. Topics Geront. 13, 239-246.
59. DUNCAN, G. and van HEYNINGEN, R. (1976) Docum. Ophthal. Proc. Ser. 8, 229-232.
60. JEDZINIAK, J.A., NICOLI, D.F., YATES, E.M. and BENEDEK, G.B. (1976) Exp. Eye Res. 23, 325-332.
61. HANNO, H.A. and WEISS, D.I. (1961) Arch. Ophthal. 65, 238-242.
62. POHJOLA, S. (1962) Acta. Ophthal. (Kbh) 40, 255-264.
63. ORTH, J., KOCH, H-R., KLEHR, H-U. and SIEDECK, M. (1978) Interdiscipl. Topics Geront. 13, 109-117.
64. BERLYNE, G.M., BEN ARI, J., DANOVITCH, G.M. and BLUMENTHAL,M. (1972) Lancet, 1, 509-511.
65. DUKE-ELDER, S. (1969) System of Ophthalmology. Vol. XI Henry Kimpton, London. p229.
66. OBARA, Y., COTLIER, E., LINDBERG, R. and HORN, J. (1976) Docum. Ophthal. Proc. Ser. 8, 193-203.
67. BROEKHUYSE, R.M. (1973) CIBA Foundation Symposium 19, 135-144.

Exp. Eye Res. (1980) **31**, 553–566

Analysis of Individual Cataract Patients and their Lenses: a Progress Report

R. M. Clayton,* J. Cuthbert,*‖ C. I. Phillips,†
R. S. Bartholomew,† N. L. Stokoe,† T. ffytche,‡ J. McK. Reid,†
J. Duffy,§ J. Seth,¶ and M. Alexander*

**Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh 9, Scotland*
†Princess Alexandra Eye Pavilion, Royal Infirmary and Department of Ophthalmology, University of Edinburgh, Chalmers Street, Edinburgh 3, Scotland
‡St Thomas's Hospital, Lambeth Palace Road, and Moorfield's Eye Hospital, City Road, London
§Department of Statistics, University of Edinburgh, West Mains Road, Edinburgh 9, Scotland
¶Department of Clinical Chemistry, Immunoassay Section, Royal Infirmary and University of Edinburgh, Bristo Place, Edinburgh 3, Scotland

(*Received 13 May 1980, New York*)

We are engaged in a continuing study of cataract patients and a control population matched for age and sex, in which epidemiological, ophthalmological, medical and other data is being collated and statistically processed by S.P.S.S. (Statistical Package for the Social Sciences).

We report some confirmation of several significant differences found previously between our cataract and control populations, including the levels of several plasma constituents and the use of certain drugs. We also report some new data, including an increased risk of cataract to individuals without clinical diabetes whose fasting blood glucose is elevated above the normal range, and also an increased risk to individuals whose blood pressure is elevated above the normal range. Protein profiles from individual cataractous lenses are compared after grouping the lenses according to the full slit lamp description and other features, and we also present a further report on some biochemical differences observed between cataracts of similar appearance but different aetiology.

Key words: human cataract; epidemiology; plasma constituents; blood pressure; specified drugs; alcohol; disease; lens protein profiles; morphopathology.

1. Introduction

In 1973, Pirie pointed out that the "almost universal description of cataracts as 'senile' without further details" hampered investigations of human lens, and went on to suggest that even one particular type of cataract (described as the "grey nuclear" cataract, which may follow posterior subcapsular opacities), could not be expected to form a homogeneous group.

Although cataract surgery is a routine ophthalmic operation it would be advantageous if preventive measures were possible for even a small proportion of cataract cases. If cataract has several possible underlying aetiologies, as is now generally assumed, then inability to discriminate validly between types of cataract will hinder the possible development of rational preventive measures or therapy.

Cataracts are generally described by morphopathology or by age of onset, and there is a very large body of literature (reviewed by Harding and Dilley, 1976) on the proteins in cataractous lenses that has firmly established the existence of age-related changes, and of

‖ In partial fulfilment of the degree of Ph.D.

0014-4835/80/110553 + 14 $01.00/0

differences in protein content and solubility between nuclear and cortical cataracts. However, investigations are normally made on proteins obtained from pooled lenses. We have previously discussed the possible loss of information which may ensue from the use of pooled lenses (Cuthbert, Clayton, Truman, Phillips and Bartholomew, 1978), even if material has been used from a group, such as nuclear cataracts, which has been further subdivided and classified, for example by nuclear colour (Pirie, 1968). It is known that certain specific groups are at especial risk of developing cataract, for example diabetics (Caird, 1973), individuals with certain G6PD variants (Zinkham, 1971), or heterozygotes for galactosemia (galactose 1 phosphate uridyl transferase deficiency) or for galactokinase deficiency (Skalka, Prchal and Conrad, 1979). Although it is still not feasible at present to predict the development of cataract in any of these individuals, it may, in principle, be a real possibility. The concept that a factor may confer liability to malfunction so that one or more other genetic or environmental conditions may also require to be present to precipitate manifest damage is fundamental to understanding specific drug susceptibilities in many human genetic conditions (for example, Vesell, 1976), and the similar concepts of 'co-cataractogenesis' and 'syncataractogenesis' have been put forward by Hockwin (1976) and Koch and Hockwin (1974) as applied to the problem of cataract.

If valid biochemical subgroups could be defined, these might be more relevant than age of onset or general morphopathology in a search for correlations between particular types of cataract with medical, ophthalmological, environmental and other factors; and also make possible a search for further groups at special risk with respect to any factors found to be cataractogenic.

Preventive or therapeutic measures have so far been offered only for such clearly characterized conditions as galactosemia and galactokinase deficiency (Gitzelmann, 1967; Gitzelmann, Curtius and Schneller, 1967), by the witholding of dietary lactose, and also for diabetic cataracts where quercitin has been tested with animal models in vitro and in vivo (Varma and Kinoshita, 1976; Beyer-Mears and Farnsworth, 1979).

The unequal distribution of the several crystallins within the lens and their differential susceptibilities to such processes as oxidation, cross-linkage or aggregation, and the existence of particular quantitative effects on crystallins in certain mutants suggested to us that cataracts with differently located lesions or different causations might be characterized by distinctive changes in the quantitative profile of crystallin polypeptide chains in both water-soluble and urea-soluble fractions (Cuthbert et al., 1978).

Lenses, fully described by slit-lamp observation, were analysed individually and their protein profiles, measured by integrating densitometry, were found to fall into several distinct groups, indicating biochemical regularities in particular forms of cataract. For example, immature, mature, and hypermature cataracts showed a clear progression of changes; the profiles of brown and yellow nuclear cataracts showed similarities in broad outline, yet were nevertheless distinct from one another. Other distinctive groups of protein profiles were found, including Down's Syndrome cataracts, juvenile, blue dot, corticosteroid cataracts, and groups with particular multiple lesions (such as blue dot coronary with posterior subcapsular opacities).

Diabetic cataracts of whatever morphopathology were found to share certain features, including elevation of some components and depression of others, which distinguished them from non-diabetic cataracts of similar morphopathology.

These data were extended in a later report (Bartholomew et al., 1980). For example it was found, using stepwise discriminant analysis, that yellow and brown nuclear cataracts form two distinct populations: either additional factors are required to produce brown

cataracts or a threshold phenomenon obtains: the occurence of diabetes-related changes in protein profiles was also confirmed. These correlations were derived from characteristics of each lens, scored for a range of values for each of 78 items of information, mostly covering levels of individual polypeptides.

Epidemiological, medical, ophthalmological and other data have been collected on individuals in the experimental and control populations. The data have been selected on the basis of evidence in the literature of association of cataracts with various genetic diseases, certain classes of drugs, occupational cataract and certain disease conditions (reviewed, Bartholomew et al., 1980). We use a questionnaire suitable for transfer to computer store, and all individuals are scored for a range of values on each of 156 items of information.

Statistically significant differences between the cataract and control populations, matched for age and sex, were found, some confirmatory of previous reports in the literature such as the higher prevalence of glaucoma, uveitis and diabetes in the cataract population, others unexpected, such as a lower prevalence of severe arcus senilis, usage of certain drugs, and significant differences in the levels of certain plasma constituents. Our previous report was based on the first 300 individuals; to date we have analysed almost 2000 individual lenses, and have fully investigated almost 900 individuals, over 100 of whom represent three special groups. We report here on some preliminary data from one such group: cataracts from a group of Korean patients recovered from leprosy, and a comparison with patients with brown or black nuclear cataracts from Sri Lanka.

2. Methods

Lenses

Individual lenses are placed on solid CO_2 in the operating theatre immediately after operation. They are stored, processed and analysed as described in Cuthbert et al. (1978) and Bartholomew et al. (1980). Lenses from Sri Lanka, the Lebanon and Korea were transported in saturated ammonium sulphate. They were homogenized and dialysed against 0·01 M-phosphate buffer, pH 7·2, containing 10 mM-mercaptoethanol, before further processing, and compared with a series of eight lenses from Edinburgh, treated and processed in the same way, as controls for this procedure. The Edinburgh lenses were a representative sample of types of cataract whose protein profiles were known to be distinctive and repeatable (Cuthbert et al., 1978).

Biochemical data

Blood was collected from patients and controls and analysed as described in Bartholomew et al. (1980).

Statistical procedures

These used the S.P.S.S. (Statistical Package for the Social Sciences) programme package (Nie, Hull, Jenkins, Steinbrenner and Bent, 1965). The numbers of patients and controls may vary for the analyses as missing values have been excluded from analysis. The tests used were t or χ^2, according to the nature of the variant being considered.

3. Results and Discussion

Cataract and control population

The current analyses are based on a total of 719 individuals (542 patients and 177 controls) since the remainder of the population is not yet fully entered into computer store.

Those plasma constituents which we reported (Bartholomew et al., 1980) as showing significant differences between the cataract and control populations are confirmed with the probability levels unchanged, or in some cases increased (Table I).

The larger population size has permitted us to analyse further the association of low plasma cholesterol with cataract (Table II). If we examine the upper and lower extremes of the distribution for serum cholesterol levels, it appears that individuals with particularly low levels (< 3·6 mmol/l) have a cataract incidence similar to those with moderate levels. However, very high levels of serum cholesterol (> 6·7 mmol/l) are associated with a significant reduction in cataract prevalence (Table II). This is presumably related to the negative correlation between cataract and arcus senilis ($P < 0.0001$, Bartholomew et al., 1980).

TABLE I

Levels of plasma constituents

Component	Patients		Controls		P
	Mean	S.D.	Mean	S.D.	
Urea (mmol/l)	6·14	2·27	5·38	1·31	< 0·0001
Plasma fasting glucose, PFG (mmol/l)	5·49	2·10	4·75	0·53	< 0·0005
Calcium (mmol/l)	2·39	0·12	2·46	0·10	< 0·0001
Albumin (g/l)	40·52	4·14	43·09	2·32	< 0·0001
Total protein (g/l)	69·40	6·14	73·12	4·59	< 0·0001
Cholesterol (mmol/l)	6·14	1·39	6·66	1·23	< 0·0001
Alanine aminotransferase (U/l)	17·21	14·10	24·56	31·89	< 0·0001
Aspartate aminotransferase (U/l)	24·68	14·36	28·51	17·66	< 0·001
Total Co_2 (mmol/l)	27·61	2·90	28·41	2·35	< 0·0011
% of individuals with PFG > 5·8 mmol/l	18·8%		3·9%		< 0·001
% of individuals with cholesterol > 6·7 mmol/l	29·8%		42·6%		< 0·005

TABLE II

Relationship between plasma cholesterol levels and cataract

Cholesterol level	Patients		Controls		Totals
	No.	%	No.	%	
< 3·6 mmol/l	16	3	2	1·1	18
3·6–6·7	363	67·2	99	56·3	462
> 6·7	161	29·8	75	42·6	236
Totals	540		176		716

$P < 0.01$.

TABLE III

Topical application of drugs

(a)

	Patients		Controls		Totals
	No.	%	No.	%	
No topically applied drugs used	477	93·0	158	99·4	635
Topical application of drugs	36	7·0	1	0·6	37
Totals	513		159		672

$P < 0.005$.

(b)

	Patients		Controls	
	No.	%	No.	%
None	447	93·0	158	99·4
Miotics	11	2·1	0	0
Adrenalin	1	0·2	1	0·6
Steroids	10	1·9	0	0
Miotics and steroids	14	2·8	0	0

In an earlier report (Bartholomew et al., 1980) we found a significant association between cataract and the topical application of drugs ($P < 0{\cdot}025$). With increased numbers in the study the significance is increased [Table III (a)]. Miotics and corticosteroids both contribute to these figures [Table III (b)].

We reported previously that we found a significantly higher usage of antihypertensive drugs among cataract patients, but no increase in cardiovascular disease. We have now assessed this data not by the presence of cardiovascular disease in general but by diastolic blood pressure levels (Table IV), excluding all those who are using antihypertensive drugs and whose blood pressure levels would be expected to be reduced (seven of 26 such users still had high levels). We set arbitrary limits for classification of diastolic blood pressure as very high and very low.

TABLE IV

Relationship between diastolic blood pressure and cataract

	Patients		Controls		
	No.	%	No.	%	Totals
Very low, < 60 mmHg	7	1·4	6	3·6	13
Median range	432	84·5	154	92·2	563
Very high, > 120 mmHg	72	14·1	7	4·2	72
Totals	511		167		678

$P < 0{\cdot}001$.

The relationship between elevated blood pressure and cataract is highly significant ($P < 0{\cdot}001$). It would seem that the use of antihypertensives does not confer significant protection against this effect, since individuals on antihypertensive medication are also highly liable to cataract (25 cases in 536 cataract patients and 1 in 168 controls), even although the blood pressure is reduced in most of them.

The Framingham epidemiological study measured numerous variables in a population over the period 1948–52. In 1973–5 this same group was investigated ophthalmologically (Kahn et al., 1977). Some senile cataract was found, the proportion rising in the older age group, as would be expected. Among the earlier measurements correlated with the later cataract data were systolic blood pressure and random blood sugar. The type of population sample and the methods of measuring blood pressure and blood sugar all differ from ours, and the elapsed time between measurements also make the results not properly comparable. Nevertheless there appears to be an agreement between this study and ours in finding an association between high blood pressure and cataract, and high blood sugar and cataract.

The mechanisms underlying the associations found may be far from direct. Kahn et al. (1977) found some association between mean casual blood-sugar level and high systolic pressure, and between both of these and ocular hypertension. These correlations, and our own population data, point to the likelihood that interactions of factors each conferring liability are very likely ultimately to emerge.

In our previous study the major contribution to the higher plasma fasting glucose levels (PFG) in cataract patients came from the diabetic subgroup. With our larger population this can be investigated more precisely, by removing the diabetic subgroup from the calculations. There is a significant excess ($P < 0{\cdot}001$) of cataracts among individuals without clinical diabetes whose fasting plasma glucose (PFG) is elevated above the normal range (> 5·8 m/mol/l) (Table V). The significant association of diabetes

with absence of a crystallin subunit, in the lens III_1 (U.S.) (Bartholomew et al., 1980) is not found in lenses from individuals with high PFG without clinical symptoms. Either the causes of these cataracts are not identical or the loss of this component is related to the duration of diabetes.

TABLE V

Plasma fasting glucose levels in non-diabetics

	Patients		Controls		
Blood glucose	No.	%	No.	%	Totals
< 5·8 mmol/l	372	86·7	99	96·1	471
> 5·8 mmol/l	57	13·3	4	3·9	61
Totals	429		103		532

$P < 0{\cdot}01$.

TABLE VI

The use of tranquillisers

	Cataract		Controls		Totals
	No.	%	No.	%	
None	445	86	146	90	591
Major	41	8	4	2	45
Minor	31	6	13	8	44
Totals	517		163		680

$P < 0{\cdot}05$.

TABLE VII

Use of alcohol

	Cataract		Control		Totals
	No.	%	No.	%	
Abstainers	217	40·6	47	27·5	264
Non-abstainers	317	59·4	124	72·5	441
Totals	534		171		705

$P < 0{\cdot}005$.

The non-diabetics with high levels of plasma fasting glucose may include pre-diabetics or occult diabetics. Falconer, Duncan and Smith (1971) estimated that the numbers of individuals whose genotype conferred liability to diabetes is higher than the number of overt diabetics. The glucose tolerance of this group might therefore be of interest, since it is possible that the mechanism of cataractogenesis in some at least of this group may be similar to that elucidated by Van Heyningen (1976) and Kinoshita (1974). A follow-up over a period of years may be required to assess how many of this group might develop clinical diabetes. Whatever the underlying situation, it would appear that high plasma glucose confers a significant risk of cataract. We hope to compare the protein profiles of cataracts from this group with those of diabetic patients in detail.

There is a higher incidence of the use of major tranquillisers (barbiturates, MAOI, tricyclics and phenothiazines) among cataract patients than among controls ($P < 0{\cdot}05$).

The users of minor tranquillisers (Valium, Librium, etc.), on the other hand, are found at similar levels in the cataract and controls groups (Table VI).

We also find some association between alcohol consumption and cataract (Table VII). Total abstainers have a higher prevalence of cataract, and non-abstainers a lower incidence, as compared to the control population ($P < 0{\cdot}005$).

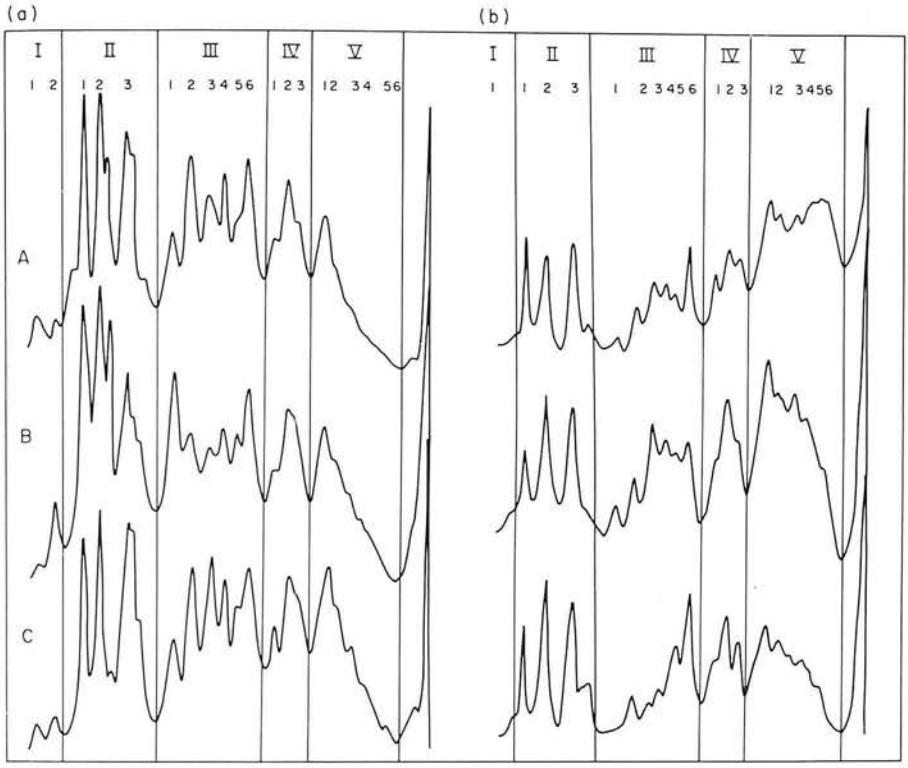


FIG. 1. Densitometer traces from the water-soluble (a) and urea-soluble (b) fractions of three cataractous lenses with white nuclear and immature cortical involvement. The preparation, electrophoresis and densitometry of the lenses was as described in Bartholomew et al (1978). (A) Cataract from Korean leprosy patient. (B) Korean lens with no leprosy history. (C) Diabetic patient, Edinburgh series. The elevation of V6 may be seen in leprosy (b)A, and the absence of III_1 in diabetes (b)C.

We record the non-abstainers according to their level of alcohol consumption, but have insufficient numbers with very high intake in the group at present to assess differences, in liability, if any, as related to alcohol consumption levels. This effect may be due to the living habits or to other biochemically unrelated factors associated with a life-style in which alcohol is avoided. More detailed analysis must be deferred.

Lenses

Of the almost 2000 individual lenses processed to date, only a small proportion have so far been analysed by stepwise discriminant analysis and other statistical procedures, but we also found that a simple comparison of the means and standard deviations for a particular component in different types of cataract may sometimes discriminate between them (Yim, 1979; Bartholomew et al., 1980). Typical traces with coded designations of the subunits are shown in Fig. 1.

The values for the subunit V_6 (Cuthbert et al., 1978) range from 0·5 to 25% of the urea-soluble fraction. A group of 34 cataract lenses from Korean ex-leprosy patients with uveitis all had values ranging from 9·5 to 25. In contrast, 26 out of 33 diabetics had values ranging from 0·5 to 9, and therefore were significantly different ($P < 0·05$) from the ex-lepers. The Korean lenses were transported to Edinburgh in saturated ammonium sulphate solution. We therefore compared these with 19 lenses with dark nuclear cataracts transported in the same way from Sri Lanka. These had V_6 values ranging from five to 17, which is similar to the values for the general population of lenses from Edinburgh. This data indicates that high levels in the lenses from the ex-leprosy patients were not due to the ammonium sulphate procedure. All Edinburgh lenses treated with ammonium sulphate as controls for this procedure show features characteristic of the cataract type, and no features peculiar to ammonium sulphate treatment were observed.

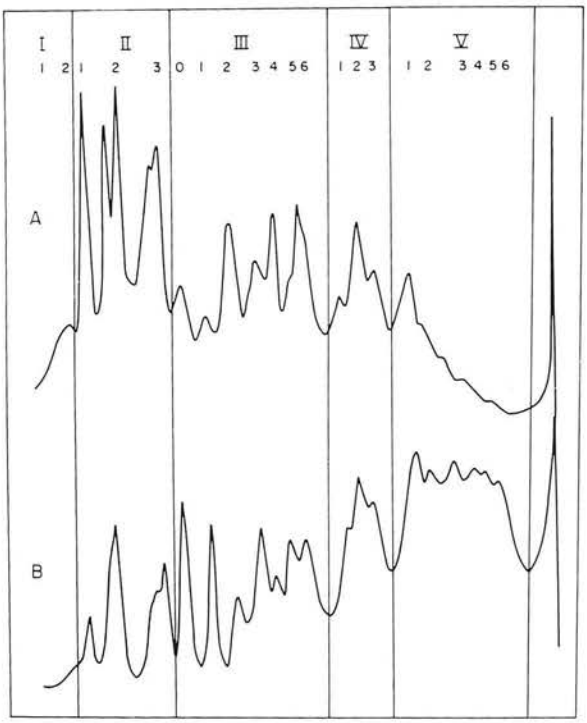


FIG. 2. The w.s. fraction of another leprosy cataract: this shows the component III_0 (found in 19 of the 33 leprosy cataracts). III_0 is also shown in the U.S. trace from a Down's Syndrome Cataract. Component III_2 is β, and V6 is cross-linked material with several specificities (Cuthbert et al., in preparation).

White nuclear cataracts occurred in 28 of 33 lenses in the Korean sample, and in one Korean lens from a non-leper. We compared these lenses with a sample of 24 white nuclear cataracts from Edinburgh. The lenses from the leprosy patients had a higher value for subunit III_2 (water-soluble fraction) than the Edinburgh group ($P < 0·01$). It may be, therefore, that the inflammatory condition in the eye had affected the amount of this component: however, we have no direct evidence to this point at present.

Another feature distinguishing the lenses from the leprosy patients is the appearance in 13 of 33 cases of material similar in mobility to III_0 (Fig. 2) previously found only in lenses from two cataracts from Down's Syndrome, three out of four cataracts from

patients with myotonic dystrophy, and a lens which had remained in a cadaver for at least 24 hr. The absence of this component from all other lenses suggests a possible breakdown product associated with severe inflammatory conditions, post-mortem change or membrane abnormality: the possibility that III_0 is such a breakdown product is open to investigation.

We suggested previously that diabetic lenses may differ from non-diabetic lenses of similar morphopathology. The data continue to support that interpretation. Subunit III_1 in the urea-soluble fraction (a β-crystallin) has the value of 0–0·5 in 21% of diabetics and in 8% of non-diabetics ($P < 0{\cdot}025$). Overall the percentage dry weight of cataracts with cupuliform involvement is not higher than that of cataracts with no cupuliform involvement. However, in agreement with our previous findings, the percentage dry weight of diabetic lenses with cupuliform involvement is higher than that of non-diabetic lenses with cupuliform involvement ($P < 0{\cdot}0005$). Thus cupuliform morphology also points to a distinction between diabetic and non-diabetic cataracts, another indication that diabetics are a special group.

4. Conclusions

As the size of the population investigated increases, it will become possible to assess the contribution to liability to cataract of a number of variables which affect only a small sample of the whole: for example, it may become possible eventually to assess the effects of particular levels of alcohol consumption or of specific drugs within a general group (the major tranquillisers) and different topically applied drugs, and eventually to assess whether any conditions are synergistic with each other.

However it is our hope that some correlations may permit the formation of hypotheses susceptible to experimental test, or to predict hazard to a particular group who may then form part of a prospective study. We also hope, in the long term, to be able to assess to what extent these associations may be more or less universally distributed and which characterise particular regions or ethnic groups.

ACKNOWLEDGMENTS

We are indebted to the following surgeons who have kindly allowed us access to their patients and have provided lenses for analysis: A. Adams, J. Hughes and G. T. Miller. It is a pleasure to acknowledge the outstanding technical assistance of Ms A. Douglas and G. Hendrey. We are glad to acknowledge the following who have offered advice or cooperation over general problems: Drs A. Doig, D. Illingworth and J. Munro and Professor J. Williamson. We are grateful to Drs M. McDonald and A. Phillips for help with the collection of lenses from the Victoria Infirmary, Kirkcaldy, and Professor A. Emery for samples from two lenses from patients with myotonic dystrophy. It is a pleasure to thank Mrs L. Dobbie for her cheerful patience with the preparation of this paper.

We are grateful to the Scottish Home and Health Department, the W. H. Ross Foundation for the Study of the Prevention of Blindness and the Iris Fund for Prevention of Blindness for their support.

REFERENCES

Bartholomew, R. S., Clayton, R. M., Cuthbert, J., Duffy, J., Phillips, C. I., Reid, J. McK., Seth, J., Truman, D. E. S., Wilson, C. and Yim, S-M. (1980). Analysis of individual cataract patients and their lenses: Preliminary observations on a population basis. In *Ageing of the Lens* (Eds Regnault, F., Hockwin, O. and Courtois, Y.). Elsevier, North Holland, Amsterdam. 241–261.

Beyer-Mears, A. and Farnsworth, P. N. (1979). Diminished sugar cataractogenesis by quercetin. *Exp. Eye Res.* **28**, 709–16.

Caird, F. I. (1973). Problems of cataract epidemiology with special reference to diabetes. *CIBA Foundation Symposium* **19**, 281–96.

Cuthbert, J., Clayton, R. M., Truman, D. E. S., Phillips, C. I. and Bartholomew, R. S. (1978). Analysis of the crystallin composition of individual human lenses: Charactistic modifications associated with different cataracts. *Interdisc. Topics Geront.* **13**, 183–92.

Falconer, D. S., Duncan, L. J. P. and Smith, C. (1971). A statistical and genetical study of diabetes. I. Prevalence and morbidity. *Ann. Hum. Genet. (London)* **34**, 347–69.

Gitzelmann, R. (1967). Hereditary galactokinase deficiency, a newly recognised cause of juvenile cataracts. *Pediat. Res.* **1**, 14–23.

Gitzelmann, R., Curtius, H.-C. and Schneller, I. (1967). Galactitol and galactose-1-phosphate in the lens of a galactosemic infant. *Exp. Eye Res.* **6**, 1–3.

Harding, J. J. and Dilley, K. J. (1976). Structural proteins of the mammalian lens: A review with emphasis on changes in development, ageing and cataract. *Exp. Eye Res.* **22**, 1–73.

Hockwin, O. (1976). Ageing of the lens and cataract. *Les colloques de l'INSERM.* **60**, 263–79.

Hockwin, O. and Koch, M. R. (1974). Combined noxious influences. In *Cataract and Abnormalities of the Lens* (Ed. Bellows, J. G.). Pp. 243–54. Grune and Stratton, Inc., New York.

Kahn, H. A., Leibowitz, H. M., Ganley, J. P., Kini, L. M., Colton, T., Nickerson, R. S. and Dawber, T. R. (1977). The Eye Study. II. Association of ophthalmic pathology with single variables previously measured in the Framingham heart study. *Am. J. Epidemiol.* **106**, 33–41.

Kinoshita, J. H. (1974). Mechanisms initiating cataract formation. *Invest. Ophthalmol.* **15**, 685–697.

Nie, N. H., Hull, C. H., Jenkins, J. C., Steinbrenner, K. and Bent, D. H. (1975). *Statistical Package for the Social Sciences.* 3rd edition. McGraw-Hill, New York.

Pirie, A. (1968). Colour and solubility of the proteins of human cataract. *Invest. Ophthalmol.* **7**, 634–50.

Pirie, A. (1973). Chairman's concluding remarks. *CIBA Foundation Symposium* **19**, 311–13.

Skalka, H. W., Prchal, J. T. and Conrad, M. E. (1979). Cataract formation in patients with decreased activity of galactosemic enzymes. *Metab. Pediat. Ophthal.* **3**, 97–101.

van Heyningen, R. (1976). Experimental studies on cataract. *Invest. Ophthalmol.* **15**, 685–97.

Varma, S. D. and Kinoshita, J. H. (1976). Inhibition of lens aldose reductase by flavanoids: Their possible role in the prevention of diabetic cataracts. *Biochem. Pharmacol.* **25**, 2505–13.

Vesell, E. S. (1976). Pharmacogenetics. In *Psychotherapeutic Drugs, Part I* (Eds Usden, E. and Forrest, I. S.). M. Dekker, New York and Basel.

Yim, S.-H. (1979). Discriminant analysis of the soluble protein composition of individual cataractous human lenses. M.Sc. Thesis, University of Edinburgh.

Zinkham, W. H. (1961). A deficiency of glucose-6-phosphate dehydrogenase activity in lens from individuals with primaquine sensitive erythrocytes. *Bull. John Hopkins Hosp.* **109**, 206–16.

Appendix

Cataract Analysis and Epidemiology: Specimen Data Collection Sheets

The computer record cards each carry a maximum of 80 items, each of which can be scored for a maximum of 12 alternatives.

Figure A 1(a), (b) shows data collection sheets 3 and 4 of Card 1: "Epidemiology and Medical Factors", which scores 80 items (sheets 1 and 2 of this card are represented in Bartholomew et al., 1980). Figure A 2(a), (b) shows data collection sheets 1 and 3 of Card 2: "Further Medical and Ophthalmological factors", which scores for 76 items. Sheet 2 of Card 2 is not shown since it repeats items in 1 and 3; containing the continuation of the right eye record and the beginning of the left eye record. Card 3 (not shown) covers clinical chemistry. It codes for 21 items, 19 of which represent different assays. This includes 65 computer entries; and in principle this could be added to in the future. At an average of three entries per substance assayed, five more plasma or other components could be added to the list. Those items, showing significant differences between patients and controls, are itemized in the text, other components are listed in Bartholomew et al. (1980). Cards 4, 5 and 6 cover lens protein analyses (water-soluble, urea-soluble and membrane components). Card 7 codes for further data pertaining to the extracted lens.

Members of our control population are fully recorded on cards 1, 2 and 3, but (obviously) not on cards 4, 5, 6 or 7.

Epidemiological: Medical

Card Number 1 [1]

Experimental Serial Number 12 13 14 15 [ ]

Status/Patient Control Relative
1 2 3 16 [ ]

Rheumatoid
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
3 Surgery
8 Other (specify)
9 Multiple 57 [ ]

Cancer
11 NA 12 UK 0 None 1 Untreated
2 Drugs (specify)
3 Surgery
4 Irradiation
8 Other (specify)
9 Multiple 58 [ ]

Immunological
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
8 Other (specify)
9 Multiple 59 [ ]

Mental & CNS
11 NA 12 NK 0 None 1 Untreated
2 Anticonvulsants (specify)
3 Surgery
8 Other (specify)
9 Multiple 60 [ ]

Respiratory
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
8 Other (specify)
9 Multiple 61 [ ]

Chronic infection (specify)
11 NA 12 NK 0 None 1 Untreated
2 Drugs (specify)
8 Other (specify)
9 Multiple 62 [ ]

Psychiatric Status
11 NA 12 NK 0 None 1 Yes
Specify e.g. senile dementia 63 [ ]

Drug History
Regular use or more than 4 months continuous use

Tranquillisers
11 NA 12 NK 0 None
1 MAOI
2 Tricyclics
3 Phenothiazines
4 Barbiturates
5 Valium, Librium (specify)
8 Other (specify)
9 Multiple 64 [ ]

Hypnotics
11 NA 12 NK 0 None
1 Mogodon
2 Choral hydrate
3 Paraldehyde
8 Other (specify)
9 Multiple 65 [ ]

Analgesics
11 NA 12 NK 0 None
1 Aspirin
2 Codeine
3 Paracetamol
4 Phenacetin
8 Other (specify)
9 Multiple 66 [ ]

Antihistamines
11 NA 12 NK 0 None
1 Yes (specify) 67 [ ]

Hormones – excluding contraceptives, corticosteroids
11 NA 12 NK 0 None
1 Yes (specify) 68 [ ]

Oral contraceptives
11 NA 12 NK 0 None
1 Yes (specify) 69 [ ]

FIG. A 1(a)

Epidemiological: Medical Page 4

Card number 1
[1]

Experiment serial number
12 13 14 15
[ ][ ][ ][ ]

Status/patient control relative
1 2 3
16 [ ]

Drug history
Corticosteroids
11 NA 12 NK 0 None
1 Systemic (oral)
2 Systemic (injected)
3 Topical
9 Multiple
70 [ ]

Antibiotics
11 NA 12 NK 0 None
1 Tetracycline
2 Frusemide
3 Isoniazid
4 Streptomycin
5 Sulphonamide
6 Penicilins
7
8 Other (specify)
9 Multiple
71 [ ]

Vasodilators
11 NA 12 NK 0 None
1 Yes, specify
72 [ ]

Bronchodilators
11 NA 12 NK 0 None
1 Yes, specify
73 [ ]

Diuretics
11 NA 12 NK 0 None
1 Yes, specify
74 [ ]

Alcohol (daily average intake)
11 NA 12 NK 0 None
1 Low, occasional use
2 Moderate (1–1½ pints/2 doubles)
3 High use (5–10 pints/½ bot.)
4 Very high use (10 pints/½ bottle daily)
75 [ ]

Tobacco
11 NA 12 NK 0 None
1 Low, occasional
2 Moderate (10 cig/d. 10z.)
3 High use (10–10/d)
4 Very high use (10/d)
76 [ ]

Self-medication
11 NA 12 NK 0 None
1 Antacids
2 Laxatives
3 Cough mixtures
8 Other, specify
9 Multiple
77 [ ]

Reproductive history
11 NA 12 NK 0 None
1 1–4 children
2 5–10 children
3 Over 10 children
78 [ ]

Age at menopause
11 NA 12 UK
1 Under 40
2 40–50
3 51–60
4 Over 61
79 [ ]

Blood pressure (diastolic)
11 NA 12 NK
1 Low 60–79
2 Normal 80–99
3 High 100–119
4 Very low < 60
5 Very high > 120
80 [ ]

FIG. A 1(b)

CATARACT ANALYSIS AND EPIDEMIOLOGY

Medical Record: Ophthalmological Record Page 1

Card number 1 2

Experimental serial number 2 3 4 5

Status: Patient 1
Control 2
Relative 3 6

Familial Data
Number of ascertainable first-degree relatives 7 8

Number of cataracts known or ascertained in first-degree relatives 9

Number of ascertainable non-consanguineous relatives (spouse, adopted sibs, adoptive parents, step parent, adoptees) 10

Number of cataracts known or ascertained in non-consanguineous relatives 11

Known genetic conditions of Individual:
Chromosomes NK 12 NA 11
None 0 Yes 1 (specify) 12

Metabolism NK 12 NA 11
None 0 Yes 1 (specify) 13

Skeleton NK 12 NA 11
None 0 Yes 1 (specify) 14

Skin NK 12 NA 11
None 0 Yes 1 (specify) 15

CNS NK 12 NA 11
None 0 Yes 1 (specify) 16

Endocrines NK 12 NA 11
None 0 Yes 1 (specify) 17

Eye NK 12 NA 11
None 0 Yes 1 (specify) 18

For patients under 10 years
Medical history of mother during pregnancy

Infection NK 12 NA 11 None 0
Yes 1 (specify) 19

Drugs NK 12 NA 11 None 0
Yes 1 (specify) 20

Illness NK 12 NA 11 None 0
Yes 1 (specify) 21

Other NK 12 NA 11 No 0
Yes 1 (specify) 22

RIGHT EYE

Glaucoma NK 12 NA 11 No 0
OA 1, CA 2, Both 3,
Secondary 4 23

Uveitis NK 12 NA 11 No 0
Anterior 1, Posterior 2, both 3 24

Macular degeneration
NK 12 NA 11 No 0 Yes 1 25

Hypertensive retinopathy
NK 12 NA 11 No 0 Yes 1 26

Diabetic retinopathy
NK 12 NA 11 No 0 Yes 1 27

Retinal detachment
NK 12 NA 11 No 0 Yes 1 28

Physical injury
NK 12 NA 11 No 0 Perforating 1
Percussive 2 Radiation 3
Multiple 4 29

Other eye conditions
e.g. (Buphthalmos
Microphthalmia
Keratoconus)
NK 12 NA 11 No 0
Yes 1 (specify) 30

Arcus
Slight 2
Mod. 3
Severe 4

FIG. A 2(a)

CATARACT ANALYSIS AND EPIDEMIOLOGY (Medical: Ophthalmological) Page 3

Refraction at presentation
60 61 62

Years between first record of cataract and operation
NK 12, NA 11, 0–1 0
2–5 1
6–10 2
11–15 3
16–20 4
20 + 5
63

Classification of cataract
(clinical appearance)
Nuclear
NK 12, NA 11, White 1, Yellow 2, Brown 3, Red/Brown 4, Black 5
64

Cortical – Anterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
65

– Posterior
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
66

– Peripheral
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
67

– Lamellar
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
68

– Sectorial
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
69

– Sutural
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
70

– Cuneiform
NK 12, NA 11, Clefts 1, Vacuoles 2, Granules 3, Iridescence 4, Multiple 9
71

Cortical-maturity
NK 12, NA 11, Early 1, Mature 2, Hypermature 3
72

Other
NK 12, NA 11, Blue dot 1, White dot 2, Coronary 3, bd. × cor. 4, bd. + wd. + cor. 7, wd. + cor. 5, bd. + wd. 6
73

Distortion of lens
NK 12, NA 11, Pseudoexfoliation 1, Displacement 2
74

Topical applications of drugs
NK 12, NA 11, None 0, Miotics 1, Adrenaline 2, Steroid 3, Other 4, (specify)
75

Date of lens extraction
NK 12, NA 11,
Right lens 1, Left lens 2
76

FIG. A 2(b)

Reprinted from:
Symposium on The Lens
Montpellier, September 20, 1980
Scientific adviser:
François Regnault
Excerpta Medica, Amsterdam-Oxford-Princeton

Cataracts: a search for associations or causative factors*

C. I. Phillips[1], R. S. Bartholomew[1], R. Clayton[2], J. Duffy[3], J. Seth[3], J. M. Reid[1], J. Cuthbert[2] and M. Alexander[2]

[1]*Department of Ophthalmology, University of Edinburgh and Princess Alexandra Eye Pavilion, Royal Infirmary;* [2]*Department of Genetics, University of Edinburgh;* [3]*Department of Statistics, University of Edinburgh; and* [4]*Department of Clinical Chemistry, Immuno-assay Section, Royal Infirmary and University of Edinburgh, Edinburgh, Scotland*

Introduction

For the past 2-3 years we have been carrying out a comparative study between cataract patients and controls from which preliminary results are now available [1, 2].

Partly because 'senile' cataract is not a single clinico-morphological entity, partly because many elderly patients have no significant cataract and partly because the disease is one of the most common causes of blindness worldwide, we decided, because of a sufficiently large number of patients, to study various aspects of the general and eye health of these 2 groups in the expectation of finding some correlates, and the hope of discovering causal factors. It was our hope that some of the causal factors would be found to be avoidable, and therefore the prevalence of cataract could be reduced. Results of analyses of crystallin subunits in cataractous lenses have already been published elsewhere [3].

**This investigation is supported by a grant from the Scottish Home and Health Department. J. Cuthbert was supported by a scholarship from the Faculty of Medicine, University of Edinburgh and has a research fellowship from the Iris Fund for the Prevention of Blindness.*

Study population

The population investigated comprised 540 cataract patients and 176 age- and sex-matched controls. Our controls consisted of members of old people's social clubs in Edinburgh and volunteers who read a newspaper account of our study.

Patients were clinically examined as in-patients 2 days before operation, and had blood samples taken (fasting) on the morning of operation. Controls either attended hospital or were examined in the morning at their social clubs one hour or so after a very light breakfast (i.e. not more than one cup of sugarless tea and one half slice of thinly buttered toast). Data were recorded on both groups and an example of one of the pages of information assembled about patients and controls is shown in the appendix.

Results

High blood pressure

This condition was more prominent in the cataract group than in the controls, excluding those patients taking antihypertensive drugs (Table I and also the appendix).

The majority of patients using antihypertensive drugs had diastolic blood pressures below 120 mm Hg yet showed a significant risk of cataract (Table II).

Table I: Diastolic blood pressure in cataract patients and controls not using antihypertensive drugs.

Diastolic blood pressure (mm Hg)	Number of cataract patients	Number of controls
<60	7 (1.4)	6 (3.6)
60-120	432 (84.5)	154 (92.2)
>120	72 (14.1)	7 (4.2)
Total	511	167

Numbers in parentheses refer to percentages.
$P<0.001$.

ıble II: Risk of cataract related to the use of antihypertensive drugs.

	Cataracts	Controls
ntihypertensive drugs	25	1
o antihypertensives	511	167

$^2 = 6.48$; $0.01 < P < 0.02$.

\ccordingly, it seems that the risk of cataract associated with high blood ressure remains even after the use of this medication.

ranquillizers

he use of major tranquillizers (barbiturates, monoamine oxidase inhibitors, ricyclic antidepressants and phenothiazines), but not minor ones (diazepam, hlordiazepoxide), was significantly associated with cataract (Table III).

Diabetes

There were significant differences, by step-wise discriminant analysis, between protein profiles of the cataracts from diabetics and of the cataracts from other patients. A very frequent feature is the absence of the III_1 (urea soluble) crystallin component from cataracts from diabetics, whereas it is very rarely absent from cataracts from non-diabetics [4, 5].

Table III: Risk of cataract related to the use of tranquillizers.

	Cataract	Control
None	445 (86.1)	146 (89.6)
Major tranquillizers	41 (7.9)	4 (2.4)
Minor tranquillizers	31 (6.0)	13 (8.0)
Total	517	163

Numbers in parentheses refer to percentages.
$P < 0.05$.

Table IV: Risk of cataract in relation to hyperglycaemia in non-diabetics.

Fasting plasma glucose	Cataract	Control
< 5.8 mmol/l	372 (86.7)	99 (96.1)
> 5.8 mmol/l	57 (13.3)	4 (3.9)
Total	429	103

Numbers in parentheses refer to percentages.
$P < 0.001$.

There was a highly significant increase in the proportion of patients with relative hyperglycaemia without clinical diabetes in the cataract population compared with controls (Table IV). In relation to the III_1 (urea soluble) crystallin component, there was no significant difference between these 2 populations.

Plasma constituents

The levels of other plasma constituents (calcium, albumin, total protein, cholesterol and total CO_2) were also estimated and were found to be considerably less in cataract patients – $P<0.0001$ in all but CO_2 where $P<0.005$. Urea levels, however, were found to be significantly higher in cataract patients ($P<0.0001$).

Discussion

How many of these significant results are independent of each other and how many are interdependent or contingent? The low plasma calcium may be partly but not wholly explained away because it depends on the level of plasma albumin. It is also interesting that there is a correlation between increasing depth of colour of the nucleus and decreasing levels of plasma calcium and albumin [2].

The association of cataract with antihypertensive drugs may not be due to

these drugs per se, but rather to the high blood pressure for which they have been prescribed. Similarly, the association with major tranquillizers may be due to some underlying condition(s) also connected with hypertension.

Although the cataract population showed low cholesterol in relation to the control population, this was actually due to the significant increase of individuals with very elevated levels in the control group.

In a small study by Goswamy et al., serum cholesterol was found to be significantly higher in each group of 8 immature cataract patients, 4 mature and 15 hypermature than in 7 normal controls [6]. Total serum phospholipids were significantly higher in the immature and mature cataract groups (not hypermature) and total serum proteins were significantly lower in each of the cataract groups. The discrepancy in the case of cholesterol between their and our results may arise from the small number of their samples and the selection of particular types of cataract.

No control group is ever perfect, just as in philosophy, arguments from analogy always break down at some point. The state of general health in the control group may have been better than that of the patients. The fasting blood sugar of the patients would be expected to be less than that of the controls, yet it was higher, which indicates that the actual association between relative hyperglycaemia and cataract may be even more significant than our figures show.

It is interesting to note that in the Framingham study population there was a significant association between relatively high systolic blood pressure at the time of the general survey, and a finding of cataract 20 years later, when an eye survey was done: the association was significant for both men and women in the age groups (at the time of the eye examination) 52-64 and 65-74, but not 75-85 years [7]. Similarly, the association between senile cataract and relative hyperglycaemia 20 years previously was significant in the 52-64 age group only, for both sexes. A significantly low vital capacity (except for males 75-85 years) and high serum phospholipid (only males 52-64 and females 65-74 and 75-85 years) were also observed.

Our future plans are of several kinds. Certain correlations cannot be investigated in sufficient detail without increasing sample size. In particular we intend to increase the numbers in our control population and also the number of patients with cataract of early onset. We suspect that the younger the cataractous patients, the more causative factors will be found, whereas the very elderly cataract patients will have relatively few. Deeper analyses of the data already obtained is now being done; for example it is possible that there is a quantitative relationship between diastolic blood pressure and the degree of risk of cataract. A search will be made for genetic factors.

References

1. Clayton, R. M., Cuthbert, J., Phillips, C. I. et al. (1980): Analysis of individual cataract patients and their lenses: a progress report. *Exp. Eye Res.* (In press).
2. Bartholomew, R.S., Clayton, R.M., Cuthbert, J. et al. (1980): Analysis of individual cataract patients and their lenses: preliminary observations on a population basis. In: *Proceedings of Symposium on Ageing of the Lens*, pp. 241-261. Elsevier/North Holland Biomedical Press, Amsterdam-Oxford-New York.
3. Cuthbert, J., Clayton, R. M., Truman, D. E. S. et al. (1978): Analysis of the crystallin composition of individual human lenses: characteristic modifications associated with different cataracts. *Interdisc. Top. Gerontol, 13*, 183-192.
4. Yim, S. H. (1979): *M. Sc. Thesis*, University of Edinburgh.
5. Duffy, J. et al. (1981): In preparation.
6. Goswamy, S., Mathur, R. L. and Agarwal, L. P. (1971): Lipoproteins of the crystallin lens and serum factors in senile cataract. *Indian J. Med. Res., 59*, 1460-1466.
7. Kahn, H. A., Leibowitz, H. M., Ganley, J. P. et al. (1977): The Framingham Eye Study II, Association of ophthalmic pathology with single variables previously measured in the Framingham Heart Study. *Am. J. Epidemiol. 106*, 33-41.

Zusammenfassung

In einer Gruppe von 540 Kataraktpatienten im Vergleich zu 176 nach Alter und Geschlecht vergleichbaren Kontrollpersonen haben wir relativ und signifikant höhere diastolische Blutdruckwerte, einen größeren Anteil von Patienten unter Antihypertonikatherapie, höhere Plasmaglukosewerte bei Nichtdiabetikern, eine größere Zahl von Patienten, die stärkere Tranquillanzien (Barbiturate, Monoaminooxidasehemmer, trizyklische Antidepressiva und Phenothiazine) einnahmen, sowie höhere Plasmaharnstoffspiegel festgestellt. Die Kataraktgruppe wies signifikant niedrigere Plasmaspiegel von Kalzium, Albumin, Gesamtprotein, Cholesterin und Gesamtkohlendioxid auf.

Appendix

Card number 1

Experiment serial number 12 13 14 15

Status: Patient/Control/Relative
1 2 3 16

Drug history
Corticosteroids
11 NA 12 NK 0 None
1 Systemic (oral)
2 Systemic (injected)
3 Topical
9 Multiple 70

Antibiotics
11 NA 12 NK 0 None
1 Tetracycline
2 Furosemide
3 Isoniazid
4 Streptomycin
5 Sulphonamide
6 Penicillins
7
8 Other (specify)
9 Multiple 71

Vasodilators
11 NA 12 NK 0 None
1 Yes, specify 72

Bronchodilators
11 NA 12 NK 0 None
1 Yes, specify 73

Diuretics
11 NA 12 NK 0 None
1 Yes, specify 74

Alcohol (daily average intake)
11 NA 12 NK 0 None
1 Low, occasional use
2 Moderate (1-4 pints or 2 doubles)
3 High use (up to 5-10 pints or up to half a bottle)
4 Very high use (more than 10 pints or more than half a bottle) 75

Tobacco (daily)
11 NA 12 NK O None
1 Low, occasional
2 Moderate (10 cigarettes)
3 High use (10-40 cigarettes
4 Very high use (more than 40 cigarettes) 76

Self-medication
11 NA 12 NK 0 None
1 Antacids
2 Laxatives
3 Cough mixtures
8 Other, specify
9 Multiple 77

Reproductive history
11 NA 12 NK 0 None
1 1-4 children
2 5-10 children
3 Over 10 children 78

Age at menopause
11 NA 12 NK
1 Under 40 years
2 40-50 years
3 51-60 years
4 Over 61 years 79

Blood pressure
11 NA 12 NK
1 Very low (60 mm Hg or less)
2 Low (61-80 mm Hg)
3 Normal range (81-100 mm Hg)
4 High (101-120 mm Hg)
5 Very high (121 mm Hg or more)

BP mm Hg 80

Pre-medication before blood taken for clinical chemistry:

NA = not applicable, NK = not known